ULTRASTRUCTURAL STUDIES ON PHYLOGENETIC RELATIONSHIPS OF THE SARCOSCYPHACEAE AND SARCOSOMATACEAE (PEZIZALES)

BY

LI-TZU LI

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1994

ACKNOWLEDGEMENTS

I would like to express my profound appreciation to Dr. James W. Kimbrough, for his guidance throughout this study. His knowledge, encouragement, valuable advice, and limitless patience have given me strong support to complete this research. I wish to extend my deep gratitude to Dr. Henry C. Aldrich for his kind suggestions and instruction of TEM techniques. I also appreciate Mrs. Jena Kimbrough for her encouragement and thoughtfulness. Sincere thanks are extended to other members of my supervisory committee, Dr. Raghavan Charudattan, Dr. Francis W. Zettler, and Dr. Saeed R. Khan for their support and guidance.

Acknowledgements are also extended to Dr. Dan E. Purcifull, director of the Electron Microscope Laboratory, for providing the use of the facilities. I also wish to thank Ms. Maureen A. Peterson for her technical assistance.

I gratefully acknowledge Dr. Gerald Benny for his encouragement, friendship, and inspiring discussions. My sincere appreciation is also directed to Drs. Chi-Guang Wu, Jaw-Fen Wang, Cherry Wang, and Esen Momol for their invaluable assistance and to my friends in Gainesville for their lovely friendship.

My very special appreciation is also extended to Mr. and Mrs. Gene Knott, my dear uncle and aunt, for their love and care for all these years.

To my parents, Chia-Tao Li and Hsiu-Hua Wu, and my brother, Yan-Chang, goes my deepest gratitude for their endless love and support. Through their love, I found strength to overcome the difficulties along the way.

Finally, I do not have enough words to express my thanks to Changshienn Wu, my husband, for his understanding and care during my graduate studies. He has become my best friend.

TABLE OF CONTENTS

		page
ACKNOWLEDGEMENTS		ii
LIST OF FIGURES		vi
ABSTRACT		xvi
CHAPTERS		
I	GENERAL INTRODUCTION	1
II	SPORE ONTOGENY OF SARCOSCYPHA SPECIES	11
	Introduction	11
	Materials and Methods	14
	Results	15
	Discussion	20
III	SPORE ONTOGENY OF PHILLIPSIA AND WYNNEA	33
	Introduction	33
	Materials and Methods	37
	Results	38
	Discussion	42
IV	SPORE ONTOGENY OF URNULA CRATERIUM	55
	Introduction	55
	Materials and Methods	57
	Results	58
	Discussion	61
V	SPORE ONTOGENY OF PSEUDOPLECTANIA NIGRELLA AND PLECTANIA NANNFELDTII	70
	Introduction	70
	Materials and Methods	74
	Results	74
	Discussion	78
VI	SPORE ONTOGENY OF GALIELLA RUFA	87
	Introduction	87
	Materials and Methods	90
	Results	90
	Discussion	93

VII SEPTAL STRUCTURES IN THE SARCOSCYPHACEAE AND SARCOSOMATACEAE 101

Introduction. 101
Materials and Methods 105
Results 106
Discussion 110

VIII SUMMARY AND CONCLUSIONS 121

BIBLIOGRAPHY 136

BIOGRAPHICAL SKETCH 144

LIST OF FIGURES

FIGURE		PAGE
2.1.	*Sarcoscypha occidentalis*. A young ascus with a diploid nucleus	26
2.2	*Sarcoscypha occidentalis* A young ascus with two haploid nuclei	26
2.3.	*Sarcoscypha occidentalis* Ascal plasma membrane in forming spore delimiting membrane	26
2.4	*Sarcoscypha occidentalis* invagination of spore delimiting membrane	26
2.5.	*Sarcoscypha occidentalis* Spore delimitation	26
2.6	*Sarcoscypha occidentalis* Detailed view of spore delimiting membrane	26
2.7	*Sarcoscypha coccinea*. Early primary wall deposition	26
2.8.	*Sarcoscypha occidentalis*. Early primary wall deposition	26
2.9.	*Sarcoscypha occidentalis*. Multinucleate spore with the primary wall	26
2.10.	*Sarcoscypha coccinea*. Multinucleate spore with the primary wall	26
2.11.	*Sarcoscypha coccinea*. Perisporic sac formation by expansion of the outer spore delimiting membrane	28
2.12.	*Sarcoscypha occidentalis* Perisporic sac formation	28
2.13.	*Sarcoscypha occidentalis*. Perisporic sac expansion at the end of the spore	28

2.14. Sarcoscypha occidentalis Accumulation of electron dense granules in the perisporic sac 28

2.15. Sarcoscypha coccinea. Accumulation of electron dense granules in the perisporic sac 28

2.16. Sarcoscypha occidentalis. Electron opaque bodies adhere to the primary wall 28

2.17. Sarcoscypha occidentalis. Epispore precursors encircle the spore 29

2.18. Sarcoscypha dudleyi. A binucleate spore with the expanded perisporic sac 28

2.19. Sarcoscypha dudleyi. Detailed view of the periplasm 28

2.20. Sarcoscypha dudleyi. Highly expanded perisporic sac filled with granules 28

2.21. Sarcoscypha coccinea. The perisporic sac and the differentiating epispore layers 30

2.22. Sarcoscypha occidentalis. Accumulation of electron dense material in the perisporic sac 30

2.23. Sarcoscypha dudleyi. Appearance of electron dense body and epispore layers 30

2.24. Sarcoscypha occidentalis. Further differentiation of the epispore 30

2.25. Sarcoscypha occidentalis. Detailed view of the perisporic sac 30

2.26. Sarcoscypha dudleyi. Electron opaque bodies appear outside of the perisporic sac 30

2.27. Sarcoscypha dudleyi. Accumulation of electron dense bodies in the perisporic sac 30

2.28. Sarcoscypha dudleyi. A large electron translucent body in the perisporic sac 30

2.29. Sarcoscypha dudleyi. Spreading of the electron translucent body 30

2.30. Sarcoscypha dudleyi. Different staining reactions of the secondary wall material and epispore layers 32

2.31. Sarcoscypha dudleyi. Fully differentiated epispore layers 32

2.32. Sarcoscypha dudleyi. Vacuolation of the periaporic sac 32

2.33. Sarcoscypha occidentalis. Degeneration of the periaporic sac 32

2.34. Sarcoscypha occidentalis. Secondary wall material deposition 32

2.35. Sarcoscypha occidentalis. Secondary wall material precipitates on the epiapora 32

2.36. Sarcoscypha occidentalis. Traces of the periaporic sac 32

2.37. Sarcoscypha occidentalis. Mature spore 32

2.38. Sarcoscypha occidentalis. Mature spore with epispore exposed 32

2.39. Sarcoscypha dudleyi. Mature spore with a layer of secondary wall 32

3.1. Phillipsia domingensis. Uneven expansion of the primary wall and vacuolate epiplasm 48

3.2. Phillipsia domingensis. Binucleate spore with electron opaque bodies on the primary wall 48

3.3. Phillipsia domingensis. The regionally expanded periaporic sac with primary wall material 48

3.4. Phillipsia domingensis. Electron opaque bodies in the epiplasm 48

3.5. Phillipsia domingensis. The primary wall deposition 48

3.6. Phillipsia domingensis. Electron opaque bodies attach on the primary wall 48

3.7 Phlillpala domingenala- Increase of electron dense granules in the primary wall. 48

3.8. Phlillpsia domingensis- Perlplasmic vesicles attach on the primary wall. 48

3.9. Philllpsia domingensis- Electron dense matrix in the epiplasm and the primary well. 48

3.10. Philllpaia domingenala- Accumulation of en electron dense matrix in the primary wall 50

3.11. Philllpaie domingenala. Two adjacent spores with the primary wall 50

3.12. Philllpsia domingensla- Detailed view of the primary wall 50

3.13. Phillipsia domingenaia- Tangential section of the spore with primary wall protrusions 50

3.14. Phillipaia domingenala- Detailed view of the protrusion 50

3.15. Phlillpsie domingensis Fnily differentiated epispore 50

3.16. Philliipala domingensia. Mature spore well. 50

3.17. Wynnea americana- The primary well and radial epispore precursors 52

3.18. Wynnea americana- Increase of the amount of epispore precursors 52

3.19. Wynnea americana- Multinucleate spore 52

3.20. Wynnea americane Dense spots in the primary well. 52

3.21. Wynnea americana Detailed view of dense spots 52

3.22. Wynnea americane- A spore with the perisporic sac 52

3.23 Wynnea americane More accumulation of epispore precursors 52

3.24 Wynnea americane Further expansion of the perisporic sac 52

3.25. Wynnea americana. The epispore 52

3.26. Wynnea americana. A spore with protrusions from the primary wall 54

3.27. Wynnea americana. Detailed view of the protrusions of the primary wall 54

3.28. Wynnea americana. The primary wall and lipid bodies in the sporoplasm 54

3.29. Wynnea americana. Detailed view of the protrusions 54

3.30. Wynnea americana. A mature spore 54

3.31. Wynnea americana. Mature spore wall 54

4.1. Urnula craterium. Early spore delimitation. 65

4.2. Urnula craterium. Early spore delimitation. 65

4.3. Urnula craterium. Completion of spore delimitation 65

4.4. Urnula craterium. Early stage of primary wall deposition 65

4.5. Urnula craterium. Detailed view of primary wall deposition 65

4.6. Urnula craterium. Perisporic sac formation. 65

4.7. Urnula craterium. The perisporic sac reduced on one end of the spore 65

4.8. Urnula craterium. Small electron dense dots on the primary wall 65

4.9. Urnula craterium. Interaction of the perisporic sac and outer spore delimiting membrane 65

4.10. Urnula craterium. Electron dense material diffuse on the inner side of the outer spore delimiting membrane 67

4.11. Urnula craterium. Detailed view of the epispore precursors 67

4.12. Urnula craterium. Pronounced precipitation of apispore precursors on the primary wall . 67

4.13. Urnula craterium. Multinucleate spore 67

4.14. Urnula craterium. Detailed view of the electron dense body..... 67

4.15. Urnula craterium. Electron dense bodies diffuse on the margin of the perisporic sac 67

4.16. Urnula craterium. The perisporic sac is filled with electron dense granules..... 67

4.17. Urnula craterium. The well differentiated apispore..... 67

4.18. Urnula craterium. The perisporic sac becomes translucent..... 69

4.19. Urnula craterium. Part of secondary wall material disintegrates..... 69

4.20. Urnula craterium. Mature spore wall... 69

5.1. Pseudoplectania nigrella. Spore delimiting membrane encircling a haploid nucleus..... 82

5.2. Pseudoplectania nigrella. Spore delimiting membrane..... 82

5.3. Pseudoplectania nigrella. Primary wall deposition occurs between the inner and outer spore delimiting membranes..... 82

5.4. Pseudoplectania nigrella. Detailed view of dense dots on the outer spore delimiting membrane..... 82

5.5. Plectania nannfeldtii. Primary wall material deposited between the inner and outer spore delimiting membranes..... 82

5.6. Pseudoplectania nigrella. The perisporic sac is filled with fragmented matrices..... 82

5.7. Pseudoplectania nigrella. Fragmented matrices seem to be epispore precursors..... 82

5.8. Pseudoplectania nigrella. Electron dense discrete matrices appear in the perisporic sac 82

5.9. Pseudoplectania nigrella. Dense matrices deposited on the primary wall as epispore precursors 82

5.10. Pseudoplectania nigrella. The amount of epispore precursors increases 84

5.11. Pseudoplectania nigrella. The perisporic sac and striated epispore 84

5.12. Pseudoplectania nigrella. The margin of the perisporic sac becomes more electron dense 84

5.13. Pseudoplectania nigrella. An electron dense body is engulfed by the outer spore delimiting membrane 84

5.14. Pseudoplectania nigrella. The dense body seems to interact with the outer spore delimiting membrane 84

5.15. Pseudoplectania nigrella. Secondary wall material in the perisporic sac 84

5.16. Plectenia nannfeldtii. Secondary wall material in the perisporic sac 84

5.17. Pseudoplectania nigrella. The well differentiated epispore 84

5.18. Pseudoplectania nigrella. The perisporic sac reduces 84

5.19. Plectenia nannfeldtii. Secondary wall material deposition 86

5.20. Pseudoplectania nigrella. Secondary wall material deposition 86

5.21. Plectenia nannfeldtii. Mature spore 86

5.22. Pseudoplectania nigrella. Mature spores 86

5.23. Pseudoplectania nigrella. Detailed view of the mature spore wall 86

5.24. Plectania nannfeldtii. Detailed view of the mature spore wall 86

6.1. Gallella rufa. Synchronous development of eight ascospores in an ascus 97

6.2. Galiella rufa. An ascus with a diploid nucleus 97

6.3. Gallella rufa. Primary wall deposition 97

6.4. Gallella rufa. Two adjacent spores with the primary wall and electron dense bodies 97

6.5. Gallella rufa. Electron translucent vesicles on the outer spore delimiting membrane 97

6.6. Gallella rufa. The outer spore delimiting membrane expands 97

6.7. Galiella rufa. The globular bodies change in electron density 97

6.8. Gallella rufa. Two types of electron dense material aggregate in the perisporic sac 99

6.9. Gallella rufa. Detailed view of the two types of electron dense material aggregating 99

6.10. Gallella rufa. Epispore precursors 99

6.11. Gallella rufa. The multinucleate spore 99

6.12. Gallella rufa. Amorphous electron opaque bodies formed by secondary wall material in the perisporic sac 99

6.13. Gallella rufa. Large discrete masses in the perisporic sac 99

6.14. Gallella rufa. Large discrete masses become homogeneous and more compact in density 99

6.15. Galiella rufa. The sporoplasm of a mature spore 99

6.16. Gallella rufa. The mature spore wall 99

7.1. Sarcoscypha occidentalis. Septal structure in the excipular cell 116

7.2. Phillipsia dominganais. Septal structure in the excipular cell 116

7.3. Wynnea americana. Septal structure in the excipular cell 115

7.4 Sarcoscypha occidentalis. Septal structure in the paraphysis 115

7.5. Sarcoscypha occidentalis. Septal structure in the paraphysis 115

7.6. Wynnea americana. Septal structure in the paraphysis 116

7.7. Sarcoscypha coccinea. Septal structure in the ascogenous hypha 115

7.8. Sarcoscypha coccinea. Detailed view of the septal structure in the ascogenous hypha 116

7.9. Phillipsia dominganais. Septal structure in the ascogenous hypha 116

7.10. Sarcoscypha occidentalis. Septal structure in the ascus 118

7.11. Urnula craterium. Septal structure in the excipular cell 118

7.12. Urnula craterium. Short cylindrical and hexagonal Woronin bodies in the excipular cell 118

7.13. Urnula craterium. Long cylindrical Woronin bodies in the excipular cell 118

7.14. Galiella rufa. Septal structure in the excipular cell 118

7.15. Galiella rufa. Long cylindrical Woronin bodies in the excipular cell 118

7.16. Galiella rufa. Septal structure in the excipular cell 118

7.17. Plectania naenfeldtii. Septal structure in the excipular cell 118

7.18. Urnula craterium. Septal structure in the paraphysis 118

7.19. Pseudoplectania nigrella. Septal structure in the paraphysis 120

7.20. Plectania nannfeldtii. An electron dense matrix in the secondary wall of the septal pore in the paraphysis 120

7.21. Urnula craterium. Septal structure in the secogenous hypha 120

7.22. Urnula craterium. Septal structure in the ascus 120

7.23. Galiella rufa. Septal structure in the ascus 120

7.24. Pseudoplectania nigrella. An electron dense matrix trapped in the secondary wall of the apical base septum 120

8.1. Comparative spore ontogeny in taxa of Sarcoscyphaceae 131

8.2. Comparative spore ontogeny in taxa of Sarcosomataceae 133

8.3. A phylogenetic scheme of Sarcoscyphineae and other families in Pezizales based upon septal structures in asci 135

Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

ULTRASTRUCTURAL STUDIES ON PHYLOGENETIC RELATIONSHIPS OF THE SARCOSCYPHACEAE AND SARCOSOMATACEAE (PEZIZALES)

by

Li-Tzu Li

April, 1994

Chairman: James W. Kimbrough
Major Department: Plant Pathology

The Sarcoscyphaceae and Sarcosomataceae in the Pezizales are characterized by having leathery or gelatinous apothecia, oblique opercula, an aporhynque type of crozier, and inequilateral ascospores. They have been placed together in the suborder Sarcoscyphineae, but differences in ascal dehiscence and pigments in their apothecia raise questions on the relationships of the two families. The purpose of this research is to investigate spore ontogeny and septal structures of selected genera in the Sarcoscyphaceae and Sarcosomataceae by using TEM techniques to help resolve these questions.

Spore wall ontogeny was found to be inconsistent and is not reliable to be used in studying phylogenetic relationships in the Pezizales. However, fine structures of the spore wall reveals useful information for solving some

xvi

taxonomic problems. The cyanophobic spore markings of the Sarcoscyphaceae are composed of the primary wall while the cyanophilous spore ornaments that commonly occur in the other Pezizales, including Sarcosomataceae, originate from the secondary wall.

Septal structures of the Sarcoscyphaceae are almost identical to those of Lachnea of the Otideaceae and are associated with globose Woronin bodies. In the Sarcosomataceae, septal pore plugs are similar to Aleuriea and are associated with various shapes of Woronin bodies, including globose, hexagonal, and long cylindrical shapes. Septal structures in asci, ascogenous hyphae, and vegetative hyphae support the idea that the Sarcoscyphaceae and Sarcosomataceae are derived from various tribes, especially Lachnea and Aleuriea, of the Otideaceae, and they are closely related to each other and to the Morchellaceae and Helvellaceae.

The roles of the parasitic nature, secal structure, nuclear condition of spores, apothecial pigmentation, spore symmetry, and spore wall structure play in evolution of the Sarcoscyphaceae and Sarcosomataceae and the other Pezizales are discussed. The Sarcosomataceae should be separated from the Sarcoscyphineae as another family in the Pezizales because of the lack of suboperculate asci.

CHAPTER I
GENERAL INTRODUCTION

Pezizales is an order of cup-fungi (Discomycetes) characterized by cylindrical, operculate asci with broadly ellipsoid, subglobose to globose, non-septate ascospores. Asci are usually formed in a hymenium. The hymenium may be reduced to a few asci or to a single ascus, resulting in small globose to discoid apothecia, or with many asci that form cupulate, saddle-shaped, gyrose or sponge-like apothecia. Early studies of classification relied mostly on features of ascocarps such as size and shape of apothecia and configuration of the hymenium. The number of families in the Pezizales that have been recognized by different authors range from as few as seven to as many as nineteen (Boudier, 1885, Seaver, 1928; Clements and Shear, 1931; Le Gal, 1947; Korf, 1954; Dennis, 1968; Rifai, 1968, Eckblad, 1968; Eriksson and Hawksworth, 1993).

In the mid-19th century, mycologists began to examine microscopic features of asci. Crouan and Crouan (1857) were the first to describe operculate dehiscence of asci. Boudier (1879) was the first to separate Discomycetes into inoperculate and operculate Discomycetes. Von Höhnel (1917) showed that a number of tropical discomycetes had tough,

leathery apothecia. Buller (1934) pointed out accentric ascal tips in some of the same genera. Seaver (1928) showed that these same genera had inequilateral ascospores as well as eccentric asci, and the asci and ascoporas were extraordinarily large. Chadefaud (1943) revealed that some of the genera of this group had an "aporhynque" type of ascal base, i.e., an atypical crozier mechanism with a trailing hyphe-like base. Later, Chadefaud (1946) examined asci and ascospores of Sarcoscyphe coccinea (Jacq.) Fr., which was initially classified as an inoperculate Discomycete. The particular disposition of the operculum of the asci which seemed to share both inoperculate and operculate characteristics, along with the presence of one-celled pluri-nucleate spores, led Chadefaud to place this fungus in the "pars-operculates," a taxon intermediate between the operculates and the inoperculates. Berthet (1964) confirmed Chadefaud's (1943) discovery and showed the aporhynque type to be present in the pars-operculates (all Sarcoscyphaceae). In addition to having the aporhynque type of ascal bases, they also had pluri-nucleate spores. The number of nuclei varies from two to twenty-five per spore depending on genera and species (Berthet 1964).

Le Gal (1946a, 1946b) examined the operculum of the pars-operculates using either dried material rehydrated with water or material preserved in formalin or in alcohol solution and found that this group possessed a "ocousinet

apical' (apical pad) and a "cousalaat apical annulaire" (apical open ring), although they were not always visible by light microscopy. Non-callose-pectic ornamented apores was another particular feature discussed by Le Gal (1946b). Le Gal distinguished three different groups according to detailed characterisitics of the apical apparatus of asci. All three groups had two layers of ascal walls, the outer layer and the inner layer. The first group, represented by Pseudoplectania, Pithya, Melascypha, Urnula, Sarcosoma, Wynnea, and Sarcoscypha, was characterized by the terminal apical chamber being located within the inner layer of ascal wall. The second group had a laterally located apical chamber between the outer and the inner ascal walls. The representatives were Phillipsia spp. The third group consisted of only Urnula geastar Peck and was similar to the second group except the outer layer of ascal wall was very thick. Le Gal (1947) proposed Sarcoscyphaceae to include nine genera of the suboperculates (corresponding to the pseudoperculates) as follows: Sarcoscypha, Cookeina, Phillipsia, Pithya, Urnula, Sarcosoma, Wynnea, Pseudoplectania, and Melascypha. Based on apothecial colors and types of tomentum, she separated this family into two tribes, the Urnuleae (dark colored apothecia) and Sarcoscyphaea (bright colored apothecia). Le Gal (1946b) believed that the operculate Discomycetes were derived from

inoparculata Discomycetes (Helotiales) through the suboparculata Discomycetes.

Arpin (1968) did a thorough study of carotenoid pigments of Discomycetes. Among the genera of Sarcoscyphaceae, Pithya, Sarcoscypha, Cookeina, Phillipsia, Urnula, and Pseudoplectania were studied. Pithya and Sarcoscypha possessed a predominant amount of either β-carotene or plectaniaxanthine or both; Cookeina and Phillipsia contained a moderate quantity of a peculiar carotenoid pigment, phillipsiaxanthine, and its derivatives. In contrast, Urnula and Pseudoplectania, which were the only genera with dark apothecia among the Urnulaceae studied, possessed no carotenoid pigments. He concluded that members of Sarcoscyphaceae (= Sarcoscyphaceae) contained various derivatives of carotenoid pigments, but members of Urnuleae had no such pigments. According to the chemical structures of the carotenoid pigments, Arpin (1968) suggested that the special pigments in the Sarcoscyphaeae were derived from β-carotene. He also proposed that the Urnuleae were derived from Sclerotiniaceae which did not have carotenoid pigments and Sarcoscypheae were derived from inoparculate Discomycetes, other than Sclerotiniaceae and Geoglossaceae, which he believed were the ancestors of Pezizineae. Berthet (1964) believed that phylogenetically the dark colored species should be considered less advanced than the bright colored ones.

Eckblad (1968) followed Le Gal (1947) in recognizing Sarcoscyphaceae, except to include Ehaedropezia, and provided a Latin diagnosis. The unique characteristics of this family stated by Eckblad (1968) included the corky or leathery but never fleshy epothecium, the gelatinous matrix in the excipulum, the apical thickening of the ascus, the oblique operculum, the inequilateral spores, and the reticulate paraphyses. Eckblad (1968, 1972) found that the suboperculate asci did have thickened apical pads and thickened opercula, but there was no apical chamber and apical open ring. He concluded that the apical chamber and apical open ring described by Le Gal (1946a, 1946b) was an artifact of fixation, since she examined only dried or pickled materials. Eckblad did not include the apical ring as a feature characterizing this family. According to the color and type of tomentum, Eckblad (1968) separated Sarcoscyphaceae into two tribes as did Le Gal, the Sarcoscyphae and the Urnulaae, to comprise eight and ten genera, respectively. Contrary to Le Gal's hypothesis (1947), Eckblad (1968) not only considered Sarcoscyphaceae advanced but also suggested that the suboperculates and the inoperculates represented parallel evolution (Eckblad, 1968, fig. 75), and that the ancestor of Sarcoscyphaceae may be extinct. He later concluded that there was no phylogenetic relationship among the inoperculates and the suboperculates because of the apparent lack of a common ancestor (Eckblad, 1972).

Rifai (1968) considered that Sarcoscyphaceae (sensu Le Gal) had sufficient sets of features to be different from the other families of Pezizales and raised Sarcoscyphaceae to a suborder. Consequently, Pezizales were split into two suborders, Sarcoscyphineae and Pezizineae. A single family, Sarcoscyphaceae, remained under Sarcoscyphineae, whereas two tribes, Sarcoscypheae and Urnuleae, were differentiated based on differences in apothecial pigmentation.

The possible phylogenetic relationships of Discomycetes suggested by Korf (1958) was that the operculates (Pezizales) were derived from the inoperculates (Helotiales) through Sarcoscyphaceae (sensu Le Gal, 1947). Subsequently, Korf (1970) proposed that Sarcoscyphaceae be divided into two families, the Sarcoscyphaceae and Sarcosomataceae, representing the tribes Sarcoscypheae and Urnuleae, respectively. The Sarcosomataceae had been proposed earlier by Kobayasi (1937) with Latin diagnosis for members of Sarcoscyphaceae (sensu Le Gal) and the type genus was Sarcosoma. However, this earlier name was ignored by both Le Gal and Eckblad. The definition was amended by Korf (1970) to accommodate the members in the Urnuleae. In addition, Korf (1970) recognized two tribes, Sarcosomateae and Galielleae, in the Sarcosomataceae and two tribes, Sarcoscypheae and Boedijnopezizeae, in the Sarcoscyphaceae. Sarcosomataceae differed from Galiellaceae by being devoid of cyanophilous spore markings. Boedijnopezizeae had spores

that mature simultaneously whereas the spores of Sarcoscyphese mature successively. Pfister (1970) studied the staining reaction of spore markings and suggested that there was probably neither callose nor pectic matrix in the spore ornaments. Rather than use "callose-pectic" as described by Le Gal (1946), "cyanophilous" was used (Kotinba and Pouzar, 1964) to describe the blue staining reaction of the spores to the dyes. Korf (1970) was also the first one that used "cysnophobic" and "cysnophilous" ornaments of the ascospores as a character in distinguishing tribes in both families.

Samuelson (1975) used transmission electron microscopy to study operculs of Sarcoscyphinese in order to clarify the structure of suboperculs, and to determine the phylogenetic relationships among Discomycetes. Based on the data obtained, he concluded that the taxa of Sarcoscyphaceae had eccentric operculs in which the ascus wall was composed of an electron-dense, enlarged inner layer, an intermediate layer, and an outer layer. On the contrary, members of Sarcosomataceae had non eccentric operculs and the ascus wall was formed by an electron-opaque, non-distinct inner layer and an outer layer. There was no evidence to support the evolution of the suboperculates from Helotiales. Furthermore, he suggested that the suboperculum was similar to that of the true operculates. Samuelson et al. (1980) discussed the direction of evolution in the Sarcoscyphinaes

and suggested that this group may be the final step in pezizalean evolution instead of being intermediate to the operculates and inoperculates.

Brummelen (1975) studied both light and transmission electron microscopic features of the ascus wall of Sarcoscypha coccinea (Scop. per S. F. Gray) Lamb. and revealed that the ascus wall consisted of three layers: an outer layer, an electron transparent middle layer, and an electron-dense, isotropic inner layer. By examining living and fixed materials, Brummelen (1975) was not able to find the apical chamber and the apical open ring as described by Chadefaud (1946) and Le Gal (1946b), and he disagreed that the suboperculates should be defined by having an apical open ring.

Another ultrastructural study of ascosporogenesis of Peziziales was done by Merkus (1973, 1974, 1975, 1976). Based upon her observations, seven types of secondary wall development of ascospores were distinguished. Among the materials studied, Sarcoscypa coccinea and Desmazieralla acicois Lib. were the only species representing Sarcoscyphaceae and Sarcosomataceae. She considered them together as the "Sarcoscypha coccinea type," according to her ultrastructural and ascospore development data, in which the secondary wall material in the perisporic sac disappeared or was only a thin layer left after the spore matured (Merkus, 1976). Donadini et al. (1989) considered

that Sarcoscypha and Pseudopithyella were similar but were two different genera and should be placed in the same family, the Sarcosomataceae, based on the ultrastructure of the ascus and ascospores. Bellemère et al. (1990) recognized Urnula helvelloides Donadini as a new genus, Donadinia, based upon subdivisions of the ascal wall layers. The new genus along with Pseudoplectania was placed in the Pseudoplectaniae, a tribe of Sarcosomataceae. Geliella was considered neither a member of the Sarcoscyphaceae nor of the Sarcosomataceae. Méndez-Howell et al. (1990) considered that Sarcoscypha, Pithya and Pseudopithyella were different based on the pattern of differentiation of the ascal wall layers.

The Sarcoscyphineae appear to be different from other Pezizales. In general, the apothecia are leathery or gelatinous but never fleshy; the asci have eccentric opercula and apothynque type of bases; the spores are inequilateral, plurinucleate, and some have cyanophobic markings which are characteristics only to this group; and the paraphyses anastomose. With those unique features, the phylogenetic relationship of Sarcoscyphineae to the other Pezizales becomes problematic and thus needs to be reevaluated.

The purpose of this dissertation is to study spore ontogeny and septal structures of the Pezizales in order to help resolve the phylogenetic relationships among the

families based upon ultrastructural characteristics. Selected genera of Sarcoscypheceae and Sarcosomataceae are investigated. In the following chapters I will discuss: Spore ontogeny of Sarcoscypha species (Chapter II), Phillipsia and Wynnea (Chapter III), Urnula (Chapter IV), Plectania and Pseudoplectania (Chapter V), Galiella (Chapter VI), the septal structures of Sarcoscyphineae (Chapter VII), and a general summary of the ultrastructure of spores and septa, and their impact on phylogeny of Sarcoscyphineae in Pezizales (Chapter VIII).

CHAPTER II
SPORE ONTOGENY IN *SARCOSCYPHA* SPECIES

Introduction

The "scarlet cup" genus, *Sarcoscypha*, is the type genus of Sarcoscyphaceae, and was initially considered to be a tribe of *Peziza* Fries (1822). Not until six decades later did Saccardo (1884) raise *Sarcoscypha* to a subgenus. Boudier (1885) then elevated *Sarcoscypha* to generic level with two species, the type species (*Sarcoscypha coccinea* (Jacq. : Gray) Lamb.) and *Sarcoscypha occidentalis* (Schw.) Sacc. Cooke (1892) recognized *Sarcoscypha* as having additional species and even included different genera currently recognized among other Pezizales. Although the genus *Sarcoscypha* is recognized by most modern authors, others chose to use *Plectania* (Seaver, 1928; Kanouse, 1948; Nannfeldt, 1949; Le Gal, 1953; Berthet, 1964).

A number of studies have contributed significantly to our understanding of the genus *Sarcoscypha*. Chadefaud (1946) and Le Gal (1946b) studied ascal structures in *S. coccinea* and described the suboperculum consisting of an apical cushion, an apical pad, an opercular cap, an apical chamber, an apical open ring, and a hinge. Le Gal (1946b) concluded that Sarcoscyphaceae had three types of opercula.

The operculum of S. coccinea belonged to the first type in her catagories. This type of ascal wall possessed two layers, the outer and the inner layers. The inner layer eventually enlarged and confined the apical chamber terminally. These peculiar ascal structures were different from those of other Pezizales and thus became known as the suboperculate type (Le Gal, 1946b). For Sarcoscypha and other genera with suboperculate asci, Le Gal (1947) proposed the family Sarcoscyphaceae. Her studies brought the suboperculate group to the attention of other mycologists. Le Gal recognized two tribes, those brightly colored taxa in the Sarcoscyphoeae, and those darkly colored ones in the Urnuleae. As noted in Chapter I, these tribes have been elevated to family rank, the Sarcoscyphae to the Sarcoscyphaceae and the Urnuleae to the Sarcosomataceae.

In a study of apothecial pigments of Discomycetes, Arpin (1968) found that S. coccinea contained 24% to 90% β-carotene and a certain amount of either torulene or plectaniaxanthine. Based on Arpin's conclusions, the Sarcoscyphae possessed carotenoid pigments in contrast to the Urnuleae which were devoid of carotenoid pigments.

Berthet (1964) revealed that the ascospores of S. coccinea were binucleate and that plurinucleate ascospores were a common feature of the suboperculate group.

Eckblad (1968) and Rifai (1968) both studied Sarcoscypha spp. morphologically and followed Le Gal's

(1946b) example in placing this genus in the Sarcoscyphaea. Eckblad (1968), who did not find the apical open ring that was described by Chadefaud (1946) and Le Gal (1946a, 1946b), considered the apical open ring to be an artifact of fixation.

Another morphological study of Sarcoscypha spp. was done by Harrington (1990). She used spore measurements, spore shape, and other features to distinguish three species, S. dudleyi (Peck) Sacc., S. coccinea, and S. austriaca (Beck ex Sacc.) Boud., from the S. coccinea complex.

Brummelen (1975) studied light and electron microscopic features of the S. coccinea ascal wall and was unable to find the apical open ring. Samuelson (1975) examined the suboperculum of the suboperculates with electron microscopy, including S. coccinea, and also came to the same conclusion.

Markus (1976) studied the ultrastructure of spores in S. coccinea and Dasmazierella acicola Lib. and found that the secondary wall material accumulating in the perisporic sac eventually disappeared. The preliminary data of ultrastructure of spore development of S. coccinea showed similarity to the other members of the Pezizales. The ultrastructural data of spore ontogeny of the other families of the Pezizales showed that the type of spore development is consistent at generic or familial levels (Dyby and Kimbrough, 1987; Gibson and Kimbrough, 1988a, 1988b;

Kimbrough and Wu, 1990; Wu, 1991, Wu and Kimbrough, 1991, 1992a, 1992b, 1993).

Rifai (1968) outlined a number of features of the Sarcoscyphaceae that appeared strikingly different from other families of Pezizales. He felt these differences warranted a separation from the Pezizales and proposed a new suborder Sarcoscyphineae. Subsequent to his work, however, the studies of Eckblad (1968), Brummelen (1975), Samuelson (1975), and Samuelson et al. (1980) raised serious questions as to Le Gal's (1946b) interpretation of the suboperculum and to the inclusion of the tribes Urnulaea and Sarcoscypheae together as a suborder (Rifai, 1968). Studies of the ultrastructure of spore wall development in members of the Sarcoscyphaceae have been limited (Markus, 1976; Donadini et al., 1989; Melendiz Howell et al., 1990). An investigation of spore ontogeny in the Sarcoscyphaceae may reveal more information on the phylogenetic relationships within the Sarcoscyphineae and to other families of the Pezizales. The purpose of this paper is to describe the ultrastructure of spore ontogeny in S. occidentalis, S. coccinea, and S. dudleyi.

Materials and Methods

Fresh specimens were processed for transmission electron microscope study following the procedures described by Carry and Kimbrough (1983). The following collections were examined: Sarcoscypha occidentalis (Schw.) Sacc.; off

Bullpen Road, 4.5 mi. S.E. of Highlands, Macon Co., North Carolina, collected by J. W. Kimbrough, on August 4, 1987, (FLAS F55272); S. coccinea (Jacq. : Fr.) Lambotte, on decaying hardwood, Watkins Mill, 5 mi. E. of Kearny, Davies Co., Missouri, collected by J. W. Kimbrough, on April 26, 1992 (FLAS F55878); and S. dudleyi (Peck) Baral, on fallen twigs of chestnut oak, Millhopper State Park, Gainesville, Alachua Co. Florida, collected by Li-Tzu Li and J. W. Kimbrough, on January 22, 1993 (FLAS F55997). Ultrathin sections were obtained on an LKB Huxley ultramicrotome with a diamond knife. After postaining with uranyl acetate and lead citrate for 30 and 10 minutes, respectively. The sections were examined at 50 kv on a Hitachi H-600 electron microscope.

Results

Early Stage of Ascosporogenesis

Early ascosporogenesis in S. occidentalis starts with a large diploid nucleus in which the synaptonemal complex is attached to the nuclear membrane in the ascus (Fig. 2.1, arrows). Meiosis I then occurs and two nuclei appear (Fig. 2.2). Subsequent meiosis II and mitosis will result in eight haploid nuclei. During meiosis, the ascal plasma membrane blebs out (Fig. 2.3) and invaginates (Fig. 2.4) to give rise to the spore delimiting membrane (SDM). The SDM, which is composed of two layers of double unit membranes (Fig. 2.6), expands and starts spore delimitation. Each

haploid nucleus is encircled by the undulating SDM (Figs. 2.5, 2.6). The same process of secosporogenesis is also found in S. coccinea and S. dudleyi. In S. coccinea, an increase of space between the SDM and sacal wall after the spore delimitation is observed (Fig. 2.7).

Primary Wall Formation

Primary wall (PW) deposition in S. occidentalis begins between the outer and inner SDMs and the primary wall is flexuous at a very early stage (Fig. 2.8). The primary wall thickens along with the increase of the space between the outer and inner SDMs, and appears electron translucent most of the time during primary wall formation (Figs. 2.9, 2.12, 2.13, 2.17), as well as in S. dudleyi (Figs. 2.18, 2.20). In S. coccinea, the primary wall appears slightly darker (Figs. 2.10, 2.11) than that of the other two species but becomes translucent later (Fig. 2.15). Although the process of mitosis is not observed, it does occur in the spores. Ascospores of S. occidentalis and S. coccinea become binucleate but are not septate at the ultrastructural level (Figs. 2.9, 2.10). Binucleate spores of S. dudleyi are found after the perisporic sac is formed (Figs. 2.18, 2.20).

Formation of the Perisporic Sac

Perisporic sac (PS) formation in S. coccinea starts at several points around the spore and the perisporic sac is electron translucent (Fig. 2.11). The outer SDM expands to delimit the perisporic sac in S. occidentalis after the

thickness of the primary wall becomes fairly uniform (Fig. 2.12). The perisporic sac is initially restricted to the area of the end of the spore (Fig. 2.13) and electron dense granular material appears as soon as the perisporic sac is formed (Figs. 2.12, 2.13). At the same time, electron dense material appears on the inner side of the primary wall (Fig. 2.14) and seems to spread toward the outer side of the primary wall (Fig. 2.16). Later, part of the perisporic sac in S. coccinea is vacuolated and others become filled with electron dense fibrillar material as in S. occidentalis (Fig. 2.15).

The perisporic sac of S. dudleyi forms more or less uniformly around the spore (Fig. 2.18), but the granularity is different from the inner to the outer area (Figs. 2.18, 2.19, 2.20). The inner area of the perisporic sac is filled with electron dense homogeneous granular material but with some small electron translucent spots (Figs. 2.18, 2.20, arrow). With higher magnification, the outer area appears reticulate (Fig. 2.19). The perisporic sac expands more in S. dudleyi (Fig. 2.20) than in S. coccinea (Fig. 2.11) and S. occidentalis (Fig. 2.13).

Differentiation of Epispore Layers

After perisporic sac formation, some electron opaque bodies (OB) appear in the perisporic sac of S. occidentalis and adhere on the primary wall (Fig. 2.16). These electron opaque bodies seem to spread out on the primary wall and

contribute to the formation of radial fibrillar epispore precursors (EPP) (Fig. 2.17). The epispore precursors of S. dudleyi are similar to those of S. occidentalis (data not shown). In S. coccinea, the electron dense granular material in the perisporic sac loses most of its density, but the radial fibrillar epispore precursors become more dense (Fig. 2.21).

The perisporic sac of S. occidentalis further dilates, more electron dense material accumulates (Fig. 2.22), and the epispore (EP) appears to increase in density (Figs. 2.22, 2.24, 2.25). The density of granules in the perisporic sac decreases (Figs. 2.24, 2.25) as well as the volume of the perisporic sac (Fig. 2.24). Electron dense material appears not only to add to the epispore, forming the first layer of the epispore, but also to spread into the primary wall (Fig. 2.25). The epispore layer later appears thicker and more fibrillar (Fig. 2.33). Epispore formation in S. coccinea is similar to that of S. occidentalis.

The epispore precursors of S. dudleyi further differentiate into the first layer of epispore and an occasional dense granular body (GB) appears in the perisporic sac (Fig. 2.23, arrow). A very thin electron dense layer appears above the first epispore layer (Figs. 2.23, 2.26). Slightly opaque bodies in the epiplasm seem to penetrate the perisporic sac (Fig. 2.26). Electron dense material accumulates to form more dense bodies in the

perisporic sac (Fig. 2.27). At the same stage, larger electron translucent bodies (TB) appear occasionally in the perisporic sac (Fig. 2.28). These large bodies later spread over the first layer of the epispore and the thin electron dense layer thickens considerably (Fig. 2.29). The outer layer of the primary wall also appears darker (Fig. 2.29). The fully differentiated epispore possesses five layers, two lightly stained layers sandwiched between three darker layers (Figs. 2.30, 2.31, 2.32).

Secondary Wall Formation

By the time the epispore of S. dudleyi is fully differentiated, there is uneven staining reaction in the secondary wall material (Fig. 2.30). The different staining reactions in the perisporic sac are not evident later, and the secondary wall material becomes uniform in granularity and density along with the maturation of the epispore layers (Figs. 2.31, 2.32). As the spore matures, the outer region of the perisporic sac becomes vacuolate (Fig. 2.32) and the secondary wall is more condensed.

The perisporic sac of S. occidentalis starts degenerating before the epispore is differentiated (Fig. 2.33). Very little secondary wall material is left on the differentiating epispore (Fig. 2.34). The fully differentiated epispore is composed of five layers, similar to those of S. dudleyi, the two light and three dark layers, and some secondary wall material remaining on the epispore

(Fig. 2.35). Traces of the collapsed perisporic sac are left on one end of the spore (Fig. 2.36). The lipid bodies located at both poles of the spore in the sporoplasm are smaller and more numerous (Fig. 2.36) than those of the completely mature spore in which the perisporic sac is totally disintegrated (Fig. 2.37).

Mature Spore Wall

Mature spore walls of S. occidentalis and S. coccineus are undulating (Figs. 2.37, 2.38), and the epispores exposed (Fig. 2.38). The mature spore wall of S. dudleyi, in contrast, is smooth and has a secondary wall layer outside of the epispore (Fig. 2.39).

Discussion

All three species of Sarcoscypha have the same process of spore deliliation and early primary wall deposition. This is typical of the other Euascomycetes (Beckett, 1973), and was clearly demonstrated in Ascodesmis nigricans van Tiegh with freeze substitution technique (Mims et al., 1980). After the primary wall reaches a certain thickness, however, S. occidentalis, S. coccineus and S. dudleyi display different types of spore wall formation. Compared with the volume of the perisporic sac, S. dudleyi has the largest perisporic sac and the most dense material within it. The perisporic sac of S. coccineus does not expand as large as in S. dudleyi and its content is very light in density with few granules. S. occidentalis possesses the most limited

expanelon of the perlsporic sac which is restricted to both ends of the spore However, the density of the periplasm appears to be intermediate between those of S. coccinea and S dudleyi

In S. occidentalis, some electron opaque bodies appear in the perisporic sac and later precipitate on the primary wall to form the radial fibrillar epispore precursors. A similar phenomenon was also found in Discina (Helvellaceae) (Kimbrough and Wu, 1990), and Mycolachnea (Otideaceae) (Wu and Kimbrough, 1992a) in which the dense bodies are much darker than those of the S. occidentalis. In Ascobolus (Ascobolaceae) (Wu and Kimbrough, 1992b), larger electron opaque bodies were found diffusing on the primary wall.

In the perisporic sac of S. coccinea, only electron dense granules appear without the appearance of electron dense bodies The granular material seems to precipitate directly onto the primary wall to form epispore precursors. This type of epispore precursor formation is seen in Peziza (Pezizaceae) (Dyby and Kimbrough, 1987), Gyromitra (Gibson and Kimbrough, 1988b), and Helvella (Helvellaceae) (Gibson and Kimbrough, 1988a)

Sarcoscypha dudleyi has the most complicated type of spore wall formation of the three Sarcoscypha species. Before the epispore is formed, the perlplasm has regions of different electron density with scattered granular bodies. Similar epispore formation was found in Cheilymenia and

Scutellinia (Otideaceae) (Wu and Kimbrough, 1991). The large translucent bodies in the perisporic sac were seen in this study previously reported in Alsuria (Otideaceae) (Wu and Kimbrough, 1993)

After ascospore precursors are formed, secondary wall material continuously accumulated in the perisporic sac of both S. occidentalis and S. coccinea and is similar to that found in species of Peziza (Dyby and Kimbrough, 1987), Gyromitra esculenta (Gibson and Kimbrough, 1988b) and Helvella (Gibson and Kimbrough, 1988a). In contrast, S. dudleyi initially has an accumulation of secondary wall material with variable electron density. That is similar to what was found in Chellymenia (Wu and Kimbrough, 1991), although Chellymenia presents a mosaic pattern of granules that is different from the more discrete pattern in S. dudleyi. Later, secondary wall material in the perisporic sac becomes uniform in density in all three species of Sarcoscypha. However, the secondary wall material of S. dudleyi gives a stronger staining reaction

In the last step of secondary wall formation, S. occidentalis and S. coccinea are similar in that the ascospore is exposed. However, in S. dudleyi some secondary wall material is deposited smoothly on the outside of the ascospore. Both types of mature spore wall were found commonly in the other Pezizales (Markua, 1974, 1975, 1976, Wu, 1991, Wu and Kimbrough, 1993)

From the process of spore wall deposition, the secondary wall seems to be built up by osmiophilic material in the perisporic sac. Of the three species, S. dudleyi is the only species that has a secondary wall. It also has the largest volume of perisporic sac and the material in the perisporic sac is the most osmiophilic. These characters seem to be related to the amount of the secondary wall that is eventually deposited on the spore wall. The perisporic sac in S. occidentalis and S. coccinea contains only a few electron dense granules even though the perisporic sac of S. coccinea is much larger. This indicates that there is not much secondary wall material in the perisporic sac and as a result a lack of secondary wall in the mature spore.

The three Sarcoscypha species studied revealed different types of spore wall ontogeny. These ultrastructural data support the morphological data (Harrington, 1990) that the S. coccinea complex should be separated into different species. Ascosporogenesis in S. coccinea is almost identical to that of Pezizaceae and Helvellaceae except devoid of a secondary wall layer. Early spore wall formation in S. occidentalis is similar to some of the Otideaceae and the later stage is similar to Pezizaceae and Helvellaceae as well as S. dudleyi. Different species in the same genus may have different types of spore wall formation. Markus (1974, 1975, 1976) also

found this in species of Trichophaea, Lasiobolus, and Pezizas

Although ascosporogenesis can be different at the species level, Sarcoscypha species appear to have various phases of spore ontogeny that are similar to that of Otideaceae, Pezizaceae and Helvellaceae. This may indicate that Sarcoscypha species are phylogenetically related to them. Furthermore, Wu (1991) suggested that Pezizaceae and Helvellaceae had the same type of spore wall formation. These data may also show that Sarcoscypha may be intermediate between the Otideaceae and the Helvellaceae.

As far as the spore wall ontogeny studied, many types of spore wall formation are found in the Pezizales. A genus that has both smooth and ornamented spores can result in different types of spore wall formation, especially secondary wall deposition (Merkus, 1974, 1975, 1976). Therefore, the spore wall ontogeny can vary from species to species. The data presented in this paper even show that a genus with only smooth spored species can have different types of spore wall ontogeny. Since ornaments of a spore originated from the secondary wall may merely be the results of ecological adaptation, spore wall ontogeny may not be useful in studying the phylogenetic relationships in the Pezizales

Figs. 2.1-2.10 Transmission electron micrographs of spore wall ontogeny in *Sarcoscypha* species.

Fig. 2.1 Diploid nucleus with synaptonemal complex (arrows) in the young ascus in *S. occidentalis* (bar = 5 μm).

Fig. 2.2 Two haploid nuclei in the young ascus in *S. occidentalis* after meiosis I (bar = 5 μm).

Fig. 2.3 Ascal plasma membrane (PM) in the very early stage of spore delimiting membrane (SDM) formation in *S. occidentalis* (bar = 0.5 μm).

Fig. 2.4 Invagination of spore delimiting (SDM) membrane from ascal plasma membrane in *S. occidentalis* (bar = 0.5 μm).

Fig. 2.5 Spore delimiting membrane (SDM) encircles single haploid nucleus (N) in *S. occidentalis* (bar = 5 μm).

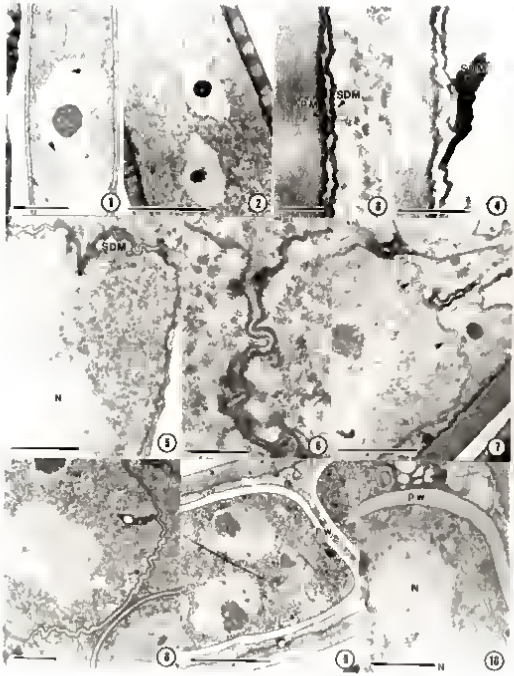
Fig. 2.6 Detailed view of spore delimiting membrane of *S. occidentalis*. Note the two-layered double unit membrane (bar = 0.5 μm).

Fig. 2.7 Early primary wall deposition between expansion of inner and outer spore delimiting membranes (arrows) in *S. coccinea* (bar = 2 μm).

Fig. 2.8 Early stage of primary wall deposition in *S. occidentalis*. Note the primary wall is flexuous (bar = 1 μm).

Fig. 2.9 Increase of thickness of the electron translucent primary wall (PW) in *S. occidentalis*. Two nuclei due to postmitosis are found in the section (bar = 5 μm).

Fig. 2.10 A binucleate spore of *S. coccinea* with more or less electron opaque primary wall (PW) (bar = 2 μm).


SDM
SDM
SDM
SDM
N
Pw
Pw
N
N
1
2
3
4
5
6
7
8
9
10

Figs. 2.11-2.20 Transmission electron micrographs of spore wall ontogeny in *Sarcoscypha* species.

Fig. 2.11 Perisporic sac (PS) formed by expansion of the outer spore delimiting membrane from several points around the spore in *S. coccinea*. Note the perisporic sac is electron translucent and the primary wall is electron opaque (bar = 2 μm).

Fig. 2.12 Perisporic sac (PS) with electron dense granules (arrows) is expanded by outer spore delimiting membrane in *S. occidentalis* (bar = 1 μm)

Fig. 2.13 Perisporic sac expands only at the end of the spore (arrow) with very limited space in *S. occidentalis* (bar = 5 μm).

Fig. 2.14 Accumulation of more electron dense granules in the perisporic sac in *S. occidentalis*. Some electron dense material (arrows) appears in the inner part of the primary wall (bar = 0.5 μm).

Fig. 2.15 Accumulation of electron dense granules in the perisporic sac in *S. coccinea*. The primary wall becomes translucent at this stage (bar = 2 μm).

Fig. 2.16 Electron opaque bodies (OB) appear in the perisporic sac and adhere to the primary wall which is deposited with more electron dense material in *S. occidentalis* (bar = 0.5 μm).

Fig. 2.17 Radial electron dense epispore precursors (EPP) encircle the spore of *S. occidentalis* with perisporic sac filled with dense granules (bar = 1 μm).

Fig. 2.18 A binucleate (N) spore of *S. dudleyi* with expanded perisporic sac with some translucent lacunae (arrow) (bar = 5 μm)

Fig. 2.19 Detailed view of granularity of the periplasm in *S. dudleyi*. The inner area of the perisporic sac is filled with uniform dense granules and the outer area appears to be reticulate (bar = 0.5 μm).

Fig. 2.20 Highly expanded perisporic sac (PS) of *S. dudleyi* filled with homogenous electron opaque granules (bar = 5 μm).

PS
PS
11
12
13
OB
14
15
16
EP
17
N
18
PS
20

Figs. 2.21-2.29 Transmission electron micrographs of spore wall ontogeny in *Sarcoscypha* species.

Fig. 2.21 The perisporic sac (PS) with few electron dense granules and a large vacuole in *S. coccinea*. The differentiating epispore layers (EP) become more electron dense (bar = 0.5 μm).

Fig. 2.22 Adjacent spores of *S. occidentalis* showing the accumulation of electron dense material in the perisporic sac after the epispore layer (EP) is formed (bar = 2 μm).

Fig. 2.23 An occasional electron dense globular body (GB) appears in the perisporic sac (PS) with uniform granularity of periplasm in *S. dudleyi*. The epispore (EP) layers becomes stratified at this stage (bar = 1 μm).

Fig. 2.24 Further differentiation of epispore with deposition of dense granules in the perisporic sac in *S. occidentalis* (bar = 5 μm).

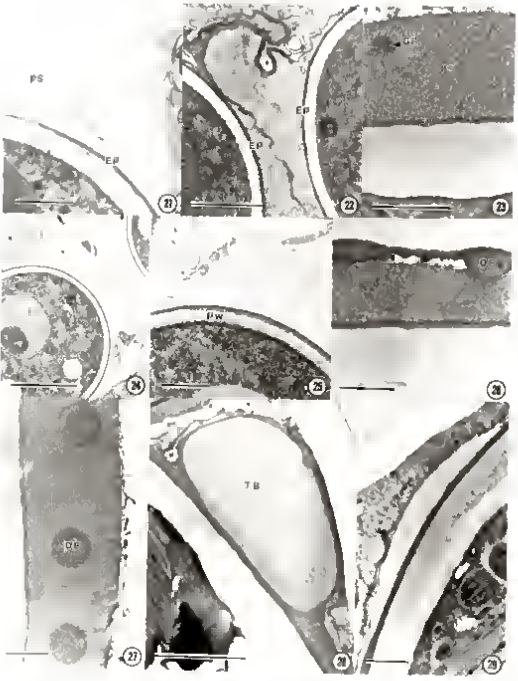
Fig. 2.25 Detailed view of the much less dense granules in the perisporic sac and penetration of dense material into the primary wall (PW) in *S. occidentalis* (bar = 1 μm).

Fig. 2.26 Electron opaque bodies (OB) appear outside of the perisporic sac and against the outer spore delimiting membrane in *S. dudleyi* (bar = 0.5 μm).

Fig. 2.27 More electron dense bodies (DB) accumulated in the perisporic sac in *S. dudleyi* (bar = 1 μm).

Fig. 2.28 A large electron translucent body (TB) within the perisporic sac of *S. dudleyi* (bar = 2 μm).

Fig. 2.29 Spreading of the electron translucent body is accompanied by an increase in thickness and electron density of the epispore layer in *S. dudleyi*. The primary wall becomes thicker and more electron opaque at this stage (bar = 1 μm).



PS
EP
21

EP
EP
22

OR
23

24

PW
25

OF
26

OP
27

TB
28

29

Figs. 2.30–2.39 Transmission electron micrographs of spore wall ontogeny in *Sarcoscypha speciosa*.

Fig. 2.30 Different staining reactions of the secondary wall material appear along with further differentiation of epispore layers in *S. dudleyi* (bar = 1 μm).

Fig. 2.31 Completely formed epispore (EP) with two lighter and three darker layers in *S. dudleyi*. Note the secondary wall material is uniformly stained again (bar = 0.3 μm).

Fig. 2.32 Vacuolation (V) of the periaporic sac occurs after epispore layers are fully differentiated in *S. dudleyi* (bar = 0.25 μm).

Fig. 2.33 The periaporic sac degenerates with the epispore not fully differentiated in *S. occidentalis* (bar = 1 μm).

Fig. 2.34 A thin layer of secondary wall (SW) material deposited on the epispore layers in *S. occidentalis*. The primary wall has electron translucent and opaque layers (bar = 0.5 μm).

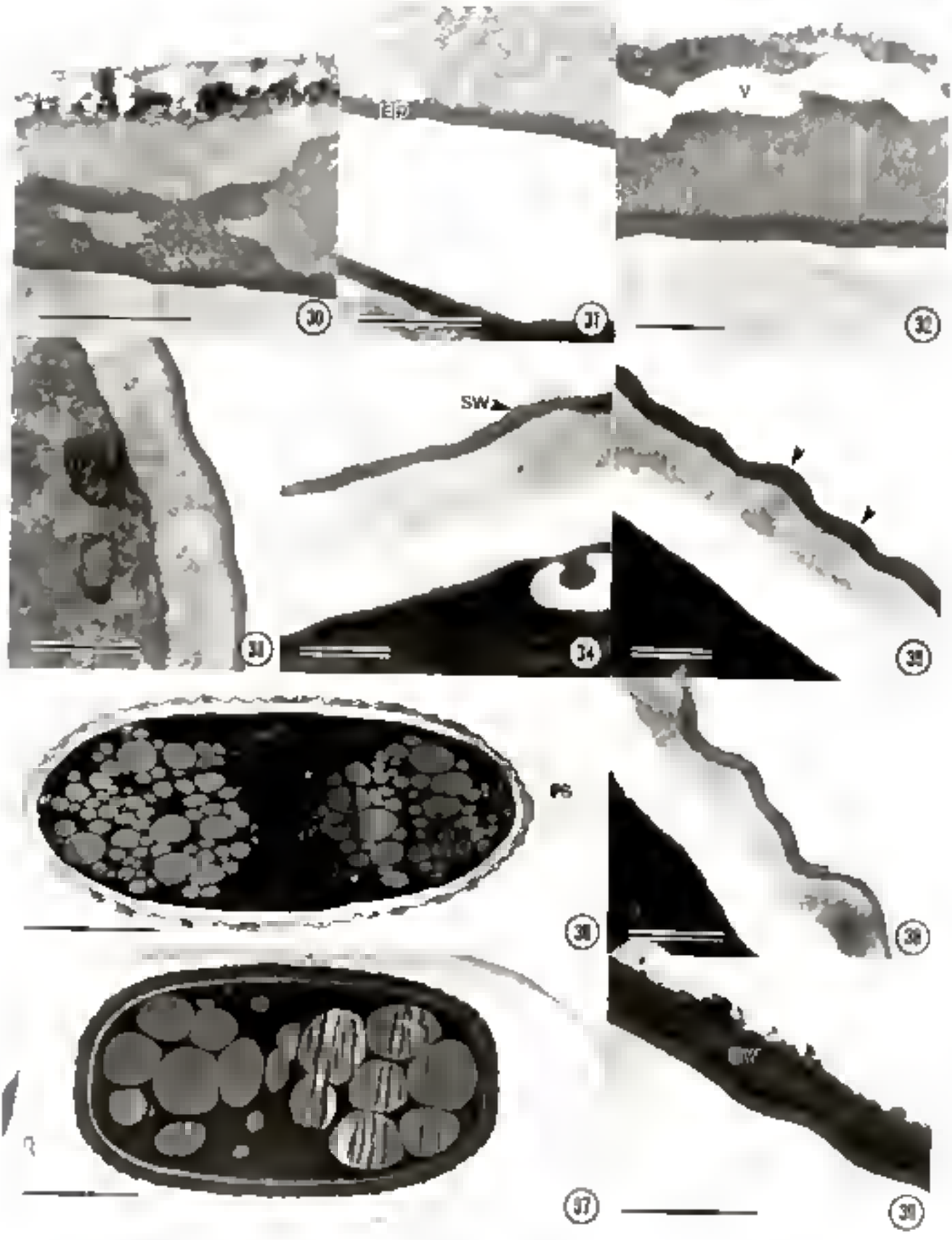
Fig. 2.35 More osmiophilic secondary wall material (arrows) precipitates on the fully differentiated epispore with two lighter and three darker layers in *S. occidentalis* (bar = 0.5 μm).

Fig. 2.36 An ascospore of *S. occidentalis* with traces of periaporic sac (PS) at one end. Note that small and numerous lipid bodies accumulate at the ends of the spore (bar = 5 μm).

Fig. 2.37 A completely matured ascospore of *S. occidentalis* with larger end fewer lipid bodies (bar = 5 μm).

Fig. 2.38 Completely matured spore wall of *S. occidentalis* with epispore exposed (bar = 0.5 μm).

Fig. 2.39 Completely matured spore wall of *S. dudleyi* with a layer of secondary wall (SW) on the epispore (bar = 0.5 μm).


V
SW
30
31
32
33
34
35
36
37
38
39

CHAPTER III
SPORE ONTOGENY OF PHILLIPSIA AND WYNNEA

Introduction

The genus Phillipsia was first described by Berkeley (1881) to include six species of brightly colored operculate Discomycetes. Seaver (1928) provided a key and descriptions for four species, including P. domingensis (Berk.) Berk. The most extensive studies of Phillipsia were done, however, by Le Gal (1946b, 1947, 1953). Le Gal (1946b) examined the operculum of several Discomycetes, including Phillipsia domingensis, the type species of the genus, and was the first to determine that it had suboperculate asci. According to Le Gal's (1946b) observations on the detailed structures of the operculum, the suboperculate group had three different types of opercula. The operculum of P. domingensis belonged to the second group in which the apical chamber was delimited between the inner and outer layers of the ascal wall and was obliquely located. One year later, Le Gal (1947) proposed the Sarcoscyphaceae to include the suboperculate group and two tribes, the Sarcoscypheae with bright colored apothecia and the Urnuleae with dark colored apothecia. She observed that Phillipsia and Wynnea Berk. & Curt. both had non-cellose pectic longitudinal ridges on

the spores. Phillipsia species were considered in the tribe Sarcoscypheae because of the bright colored apothecium and hymenium (Le Gal, 1953). Her taxonomic scheme was adopted by a number of subsequent authors (Arpin, 1968, Rifai, 1968, Eckblad, 1968; Denison, 1969, Korf, 1973).

In Berthet's (1964) cytological study of Discomycetes, P. domingensis was found to have eight to twelve nuclei in each ascospore. The multinucleate spore is now considered one of the features of the suboperculate group.

A thorough examination of carotenoid pigment contents of Discomycetes was done by Arpin (1968), who examined three species of Phillipsia: P. carminea (Fat.) Le Gal, P. subpurpurea Berk. et Br., and P. carnicolor Le Gal all possessed more than 70% of phillipsiaxanthine, a more elaborate carotenoid pigment derived from β-carotene.

Rifai (1968) recognized three series within Phillipsia. The first, represented by P. domingensis and P. subpurpurea, had large apothecia with a well developed medullary excipulum. The second, P. minor (Wakef.) Rifai, had small apothecia with a thin medullary excipulum. The last, characterized by P. umbilicata (Penz. & Sacc.) Boedijn, had apothecia like P. minor, but the asci did not have a flexuous, narrow and hypha-like base. Rifai (1968) suggested that the third series was quite different from the other two and proposed a new genus, Aurophora Rifai, to accommodate species of Phillipsia which had fan-shaped

apothecia and a gelatinous matrix in the medullary layer. Based on morphological, cytological, and biochemical data, Rifai (1968) elevated the Sarcoscyphaceae to subordinal rank, the Sarcoscyphineae.

Korf (1970) later separated Sarcoscyphineae into two families, the Sarcoscyphaceae and the Sarcoromataceae to accommodate the Sarcoscyphaeae and the Urnuleae, respectively. Phillipsia was placed in the Sarcoscyphaceae tribe Sarcoscypheae in which the spores mature successively.

The first ultrastructural study of Phillipsia was done by Samuelson (1975) who examined the operculum of P. dominganeia and found the operculum to be eccentric like that in Sarcoscypha and Wynnea. Benny and Samuelson (1980) found that the septal pore of P. dominganeia vegetative hyphae was similar to that of the other Ascomycetes which were plugged by Woronin bodies.

Wynnea, erected by Berkeley and Curtis (Berkeley, 1867), has rabbit ear-like, fasciculate apothecia and non-callose-pectic (cyanophobic) longitudinal spore markings. Two species, Wynnea macrotis (Berk.) Berk. and Wynnea gigantea Berk. & Curt. (the type species), were included. Thaxter (1905) described a third species, W. americana. Thaxter, in which hypogeous sclerotia gave rise to apothecia. Later, Le Gal (1946b) studied the operculum of Wynnea americana along with other Sarcoscyphaceae, and first recognized it as a member of the suboperculate group. Le

Gal (1946b) described W. americana with an operculum in which the terminal apical chamber was in the inner layer of the ascal wall.

The taxonomy of Wynnea had been problematic because of the apothecial color. Korf (1949) first mentioned that the fresh young apothecium and hymenium of this fungus were pinkish and turned quite dark when they aged. Korf's description was overlooked, apparently, because Eckblad (1968), Rifai (1968) and Denison (1969) placed Wynnea in the Sarcoscyphaceae (sensu Le Gal) tribe, Urnulese, because of its dark-colored apothecium and hymenium. Korf later (1970) included Wynnea in the Sarcoscyphaceae (=Sarcoscyphaceae) instead of the Sarcosomataceae (=Urnulese) based upon the bright color of fresh young apothecia. In the monograph of Wynnea, Pfister (1979) agreed with Korf's treatment of this genus and mentioned that Wynnea was the only genus found in Sarcoscyphaceae which was not associated with woody plants or leaves.

The non-calloso-pectic spore markings of both Phillipsia and Wynnea are one of the unique features of most Sarcoscyphaceae. Rather than use "calloso-pectic" as described by Le Gal (1946b, 1947, 1953), "cyanophilous" is used to express the blue staining reaction of the spore markings (Kotlaba and Pouzar, 1964; Korf, 1970). Furthermore, it was proven by Pfister (1970) that there was neither callose nor pectin in fungi. Korf (1970) used

"cyanophobic" and "cyanophilous" ascospore ornaments as a character to distinguish tribes of both Sarcoscyphaceae and Sarcosomataceae.

Despite the lack of cytochemical data on carotenoid pigments, Wynnea has been shifted between the Sarcoscyphaceae and the Sarcosomataceae. The cyanophobic spore ornaments seem to play an important role in determining the relationship of Wynnea to the proper family (Sarcoscyphaceae) and to the genus Phillipsia. The purpose of this paper is to describe the ultrastructure of ascospore ontogeny in both Phillipsia and Wynnea and to provide information that can reveal phylogenetic relationships of Phillipsia and Wynnea to families of Sarcoscyphaceae and Sarcosomataceae and to the other families in the Pezizales.

Materials and Methods

Field specimens were processed for transmission electron microscope study following the procedures described by Curry and Kimbrough (1983). The following collections were examined: Phillipsia domingensis (Berk.) Berk., S. W. 20th Avenue, near I-75, Gainesville, Alachua Co., Florida, on September 16 1983, (FLAS F53645); Wynnea americana Thaxter; (unassessioned), Bell Creek Road, Hydrology Research area, Coweeta, Macon Co., North Carolina, on August 14 1987, (FLAS F55286), on August 14 1992, (FLAS F55994). Ultrathin sections were obtained on an LKB Huxley ultramicrotome with a diamond knife. After postfixing

with uranyl acetate and lead citrate for 30 and 10 minutes, respectively, the sections were examined at 50 kv on a Hitachi H-600 electron microscope.

Results

Phillipsia domingensis

The early stage in the formation of spore delimiting membrane (SDM) was not observed in *P. domingensis*. The earliest stage examined was the delimitation of young haploid uninucleate (N) ascospores with unevenly thick, flexuous and electron translucent primary wall (PW) formed between the outer and inner SDMs (Fig. 3.1, arrows). The epiplasm (EPM) in the young ascus seems to condense, becomes electron dense and highly vacuolate, and some of it tightly surrounds the primary wall (Fig. 3.1).

Mitosis occurs in the ascospore after primary wall deposition begins and results initially in a binucleate spore (Fig. 3.2). The epiplasm around the ascal wall at this time appears less dense, more vacuolate, and electron opaque bodies (OB) adhere on the primary wall leaving the other areas of the ascus electron translucent (Fig. 3.2). Subsequent primary wall deposition results as opaque bodies left by the vacuolate epiplasm, which appear to be trapped between the two layered outer SDM of adjacent spores (Figs. 3.3, 3.4, 3.5). The perisporic sac (PS) at this stage has only enough space for opaque bodies. The primary wall expands locally with some dense granules inside (Fig. 3.3,

arrow). More opaque bodies produced in the epiplasm seem to penetrate into the perisporic sac and are accompanied by further elongation and increase in electron dense granules of the primary wall (Fig. 3.4).

With the disappearance of electron dense granules, the primary wall becomes electron translucent and increases in thickness (Fig. 3.5). More electron opaque bodies appear to be trapped (Fig. 3.5) and pass the outer SDM to reach the primary wall (Fig. 3.6, arrow). During primary wall deposition, the sporoplasma membrane becomes very undulate (Figs. 3.3, 3.4, 3.6, 3.7) and sometimes forms an elongated sac protruding deeper into the sporoplasm (Fig. 3.6). Adherence of the opaque bodies to the primary wall seems to add much more wall material and to increase the amount of electron dense granules in the primary wall (Fig. 3.7).

In addition to electron opaque bodies, less dense vesicles also appear and attach to the primary wall (Fig. 3.8, arrow). Another electron dense globular matrix (DM) is attached from the epiplasm to the primary wall (Fig. 3.9). A similar electron dense matrix later accumulates inside the primary wall (Fig. 3.10). Subsequent mitosis occurs during primary wall formation and several nuclei may be observed in thin section (Fig. 3.11). At the same time, the primary wall becomes electron dense (Fig. 3.11). With higher magnification, the primary wall actually has different staining reactions in which the inner layer is electron

translucent and the outer layer electron dense and granular (Fig. 3.12).

In tangential section, an ascospore appears inequilateral with a large lipid body (L) occupying most of the volume (Fig. 3.13). The longitudinal ridges appear as protrusions originated from primary wall material (Figs. 3.13, 3.14, 3.16). Zonation of the primary wall becomes more apparent at this stage. The inner electron translucent zone expands considerably and the outer dense zone is darker and becomes homogeneous (Figs. 3.13 - 3.16).

The perisporic sac becomes more obvious and is filled with secondary wall material when the epispore is formed (Fig. 3.14). Epispore precursors were not observed. The fully differentiated epispore (EP) is composed of two thin dark layers and one light intermediate layer (Figs. 3.14, 3.15). The perisporic sac degenerates when the spore matures and there is no secondary wall on the outside of the epispore (EP) (Fig. 3.15, 3.16). Mature spore ornaments are formed by the dense zone of the primary wall and the spore is encircled by the epispore (Fig. 3.16).

Wynnea americana

Early stages of ascosporogenesis were not observed. After spore delimitation and early primary wall (PW) deposition, radial electron dense epispore precursors (EPP) with an occasional dense body (DB) appear on the electron translucent primary wall, and the periaporic sac (PS) is

filled with loose electron dense fibrils (Fig. 3.17). The dense bodies, as well as the fibrils in the perisporic sac, disappear and the amount of spispore precursors increases slightly (Fig. 3.18). At the same time, some short electron dense bands appear on the outer spore delimiting membrane (SDM) (Figs. 3.18, 3.23).

Mitosis occurs in the young ascospore and as a result several nuclei (N) with large condensed chromatin bodies are found in the section (Fig. 3.19). The epiplasm (EPM) in the secus is translucent with only a small amount of granular material (Figs. 3.19, 3.22, 3.26). Later, some dense spots appear in the primary wall (Fig. 3.20). Higher magnification of the section reveals that the dense spots are the extension of the sporoplasma membrane from the sporoplasm into the primary wall (Fig. 3.21, arrow).

Further expansion of the perisporic sac (Fig. 3.22) is accompanied with more accumulation of the radial spispore precursors and with dense material that spreads into the primary wall (Fig. 3.23). A considerably expanded perisporic sac is filled with electron dense fibrils, and numerous dense globular bodies (GB) adhere to the edge (Fig. 3.24). Afterwards, the dense fibrils in the perisporic sac become parietinal and the spispore precursors are composed of both radial and parietinal fibrils (Fig. 3.25).

Small protrusions originating from the translucent primary wall are formed on the spore wall (Figs. 3.26, 3.27)

and organelles in the sporoplasm are clearly seen at this stage (Fig. 3.26). The periplasm remains reticulate in some areas, and a dark zone appears in the primary wall next to the epispore (Fig. 3.27). When the spore matures, the lipid bodies (L) inside become much larger and occupy most of the sporoplasm (Fig. 3.28). The primary wall appears electron opaque and thickens unevenly with enlargement of the small protrusions (Fig. 3.28, arrow). Enlargement of the protrusions, representing the longitudinal ridges of the spore is more prominent on the end of the spore. The perisporic sac disintegrates simultaneously (Fig. 3.29). The spore becomes more osmophilic during maturation (Fig 3.30). The primary wall of mature spores is translucent in the inner side with a thin outer dense zone, and the epispore (EP) is exposed without any secondary wall layer (Fig. 3.31)

Discussion

Many Sarcoscyphaceae have more or less synchronous spore development and will not form apothecia in culture. Therefore, since only field material was available only limited stages were fixed for TEM. The origin of primary wall material has been discussed by other authors. Markus (1976) mentioned that the sporoplasma membrane resulting from the inner spore delimiting membrane might play a role in primary wall development, and that the outer spore delimiting membrane might be involved in both primary and

secondary wall formation. Although the sources of wall material were not directly suggested by Merkus, she seemed to indicate that the primary wall material was derived from the sporoplasm and epiplasm, whereas the secondary wall material was from the epiplasm. Similar implications were also made by Beckett (1981). However, without definitive stains or immunocytochemical techniques, it will be difficult to determine the source or sources of wall compounds. Through the analysis of electron micrographs, the staining property and physical nature of wall materials enable one to speculate the origin of various wall materials.

In the ultrastructural study of Heivella, Gibson and Kimbrough (1988a) suggested that primary wall material was possibly derived from both the epiplasm and sporoplasm although there was no evidence to support this. Kimbrough and Wu (1990) studied Discina and suggested that the primary wall was synthesized in the sporoplasm because of the existence of numerous mitochondria, vesicles, and dense cytoplasm. A series of data from other genera of Pezizales, including sporoplasmic vesicles found in Tarzetta, Trichophaea (Wu, 1991), and Ascobolus (Wu and Kimbrough, 1992b), and sporoplasma membrane-associated dense bodies found in Mycolachnea (Wu and Kimbrough, 1992a), indicated that the sporoplasm may contribute to primary wall formation.

Primary wall formation of P. domingensis is much more complicated than any other Fazizalaa that have been studied. The undulated sporoplasma membrane and elongated sac protruding to the sporoplasm indicate that the sporoplasm may be one of the sources of primary wall material. Based upon observations of other genera (Chapter II, Chapter IV, Chapter V, Chapter VI), the electron opaque bodies and the electron dense globular matrix very possibly originate from the epiplasm and are later deposited upon the primary wall. The electron dense bodies, because of their staining property, may be the major component of the dense zone of the primary wall. The vesicles that attach to the primary wall may also carry some electron translucent material from the epiplasm to contribute to the translucent zone of the primary wall. The ultrastructural data indicated that both the epiplasm and sporoplasm contribute to the primary wall.

In W. americana, early stages of primary wall formation were not found. However, in the later stages, sporoplasma membrane extends from the sporoplasm to the primary wall. This indicates that there is an interaction between sporoplasm and the primary wall during wall development and that the sporoplasm may be the source of the primary wall material.

Perisporic sac expansion is not obvious in P. domingensis at early stages of spore wall formation. After spore markings are formed, however, the perisporic sac

becomes more distinct. In contrast, W. americana has a more widely expanded perisporic sac and it does not collapse until spore ornaments are formed. The late expansion of the perisporic sac may be evidence that final primary wall formation also relies on material in the perisporic sac.

The process of epispore formation was not observed in P. domingensis. In W. americana, epispore formation is similar to what was found in Mycolachnea (Wu and Kimbrough, 1992a), Sphaerosporella (Wu, 1991), and Aleurla and Octospora (Wu and Kimbrough, 1993), which are members of the Otideaceae.

Beckett (1981) pointed out that most of the spore ornaments were from secondary wall material. However, the cyanophobic longitudinal ridges of P. domingensis and W. americana originate from the primary wall and there is no secondary wall material on the outside of the epispore. So far, there are no examples found in the Pezizales other than Sarcoscyphaceae that have spores with either cyanophobic markings or spore markings composed of primary wall material. The cyanophobic ridges on spores of the Sarcoscyphaceae reflect the inability of Pezizales primary walls to absorb cotton blue dyes. In contrast, the ornaments originating from secondary wall material stain blue. Pithya, another genus in Sarcoscyphaceae that has smooth spores, was found by Malendez-Howell et al. (1990) to have minute ornaments which were not visible under the light

microscope. Even though the authors did not specify the origin of the ornamenta, it is apparent that the ornamenta are composed of primary wall material.

In many cases spore wall ontogeny may not provide reliable evidence for studying phylogenetic relationships above species level because of its inconsistency. However, the process of primary wall synthesis observed in both Phillipsia and Wynnea is quite different from that of the other families of Pezizales. The longitudinal ridges with primary wall material origin found in W. americana and P. domingensis are so unique that they can be used to distinguish Sarcoscyphaceae from the other families of Pezizales. Based upon Korf's (1949) description on Wynnea with bright colored apothecia when young and fresh, and the unique ultrastructural features that are found in both Phillipsia and Wynnea, the latter should remain in the Sarcoscyphaceae.

Figs. 3.1-3.9 Transmission electron micrographs of spore wall ontogeny in *Phillipsia domingensis*

Fig. 3.1 Uneven expansion of the translucent primary wall (PW) between the outer and inner spore delimiting membranes (arrows). The epiplasm (EPM) is electron dense and highly vacuolate (bar = 2 μm).

Fig. 3.2 Binucleate (N) ascospore with electron opaque bodies adhering on the primary wall (arrows). The remaining epiplasm appears less electron dense (bar = 5 μm).

Fig. 3.3 The perisporic sac (PS) with primary wall material expands regionally with electron opaque bodies (arrows) trapped between adjacent perisporic sacs (bar = 2 μm).

Fig. 3.4 Electron opaque bodies (OB) produced in the epiplasm seem to move into the perisporic sac. Note the appearance and elongation of electron dense granules in the primary wall (bar = 0.5 μm).

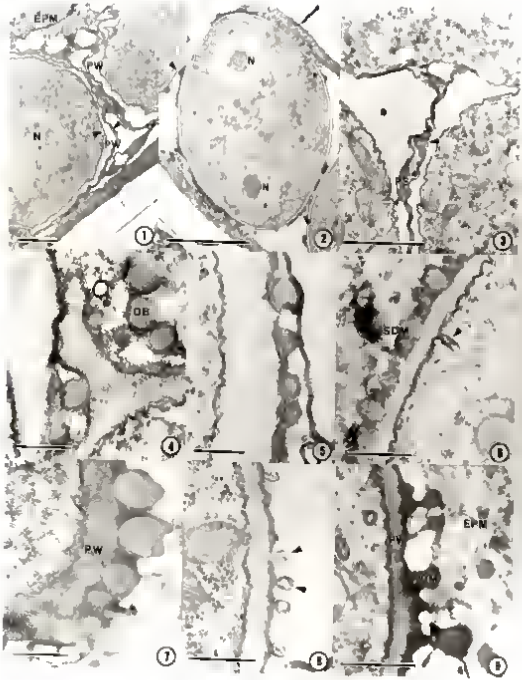
Fig. 3.5 The primary wall increases in thickness and becomes electron translucent. More electron opaque bodies remain trapped in the perisporic sac (bar = 0.5 μm).

Fig. 3.6 Electron opaque bodies penetrate through the outer spore delimiting membrane (SDM) to attach on the primary wall. The sporoplasma membrane is undulate and forms an elongated sac protruding (arrow) into the sporoplasm (bar = 0.5 μm).

Fig. 3.7 Increase of electron dense granules in the primary wall (PW) with penetration of electron opaque bodies (bar = 0.5 μm).

Fig. 3.8 Periplasmic vesicles (arrows) attach on the primary wall (bar = 0.5 μm).

Fig. 3.9 Electron dense globular matrix (DM) extending from the epiplasm (EPM) to the primary wall (PW) (bar = 0.5 μm).


EPM
PW
N
PW
N
N
PS
1
2
3
OB
SDM
4
5
6
PW
EPM
7
8
9

Figs. 3.10-3.16 Transmission electron micrographs of spore wall ontogeny in *Phillipsia domingensis*

Fig. 3.10 Accumulation of an electron dense matrix (DM) in the primary wall (bar = 0.5 μm)

Fig. 3.11 Two adjacent spores with the electron dense primary wall. One of the spores has three nuclei (N) (bar = 5 μm).

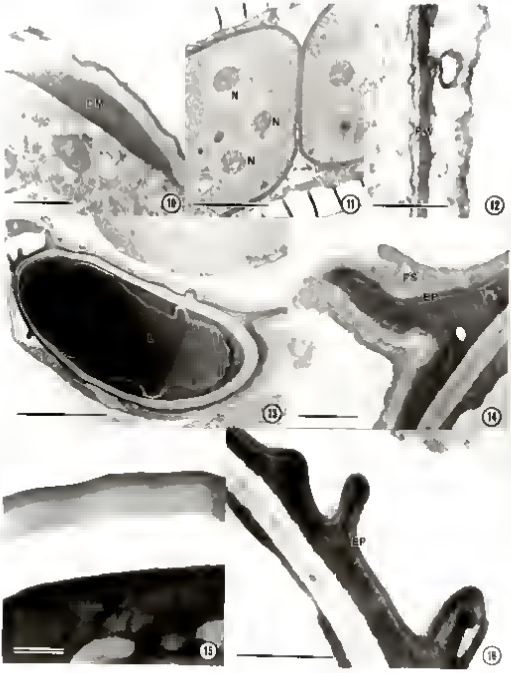
Fig. 3.12 Detailed view of the primary wall (PW) with one inner electron translucent and an outer electron dense zone (bar = 1 μm).

Fig. 3.13 Tangential section of an ascospore with one large lipid body (L) in the sporoplasm. The primary wall has an inner translucent zone and an outer electron dense zone that gives rise to spore wall protrusions (bar = 5 μm)

Fig. 3.14 Detailed view of the spore wall showing a rib or protrusion that is an extension of the electron dense layer of the primary wall. The primary wall protrusion is separated from the dense granular perispore (PS) by a sonste epispore layer (EP) (bar = 0.5 μm)

Fig. 3.15 Fully differentiated epispore is composed of two thin dark layers and one light intermediate layer. The perisporic sac is degenerated (bar = 0.5 μm)

Fig. 3.16 Mature spore with the protruding primary wall encircled by the exposed epispore (EP) (bar = 0.5 μm).


PM
N
N
N
PM
PS
EP
EP
10
11
12
13
14
15
16

Figs. 3.17-3.25 Transmission electron micrographs of spore wall ontogeny in *Wynnea americana*.

Fig. 3.17 The electron translucent primary wall (PW) with radial epispore precursors (EPP) and electron dense body (DB) deposited on the wall. The perisporic sac (PS) is filled with loose electron dense fibrils (bar = 1 μm)

Fig. 3.18 Increase of the amount of epispore precursors is accompanied by the disappearance of electron dense fibrils in the perisporic sac. Some dense bands (arrows) appear on the outer spore delimiting membrane (SDM) (bar = 0.5 μm).

Fig. 3.19 Longitudinal section of a tetranucleate (N) ascospore with condensed chromatin in the nuclei. The epiplasm (EPM) in the ascus is translucent with only a small amount of granular material (bar = 5 μm).

Fig. 3.20 Dense spots are found in the translucent primary wall (bar = 2 μm)

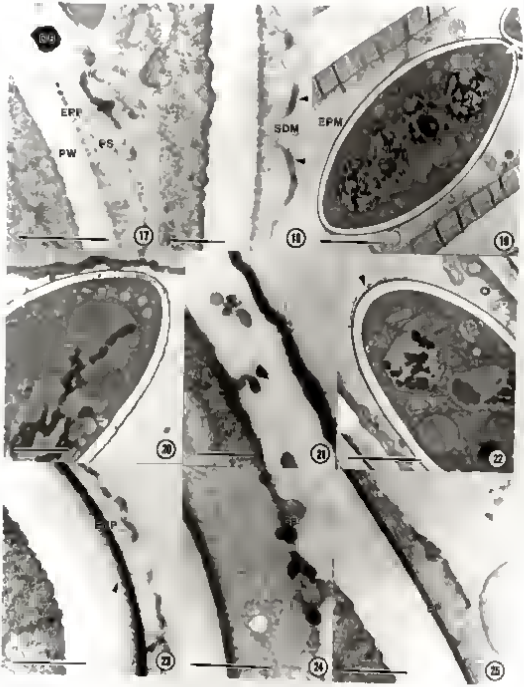
Fig. 3.21 Detailed view of dense spots in the primary wall. The sporoplasma membrane extends from the sporoplasm into the primary wall (arrow) (bar = 0.2 μm).

Fig. 3.22 Slightly more mature spore with a thin expanded perisporic sac (arrow) (bar = 5 μm).

Fig. 3.23 More accumulation of epispore precursors (EPP) and spread of dense material (arrow) into the primary wall (bar = 0.5 μm)

Fig. 3.24 Further expansion of the perisporic sac filled with dense fibrils and some dense globular bodies (GB) on the edge (bar = 0.5 μm)

Fig. 3.25 The epispore (EP) is composed of radial and pericilinal fibrils. The dense fibrils in the perisporic sac becomes pericilinal (bar = 0.5 μm).


EPP
PS
PW
SDM
EPM
17
18
19
20
21
22
23
24
25

Figs. 3.26-3.31 Transmission electron micrographs of spore wall ontogeny in *Wynnea americana*.

Fig. 3.26 Cross section of an ascospore with organelles visible in the sporoplasm. Some small protrusions originate from the translucent primary wall (PW) (bar = 5 μm).

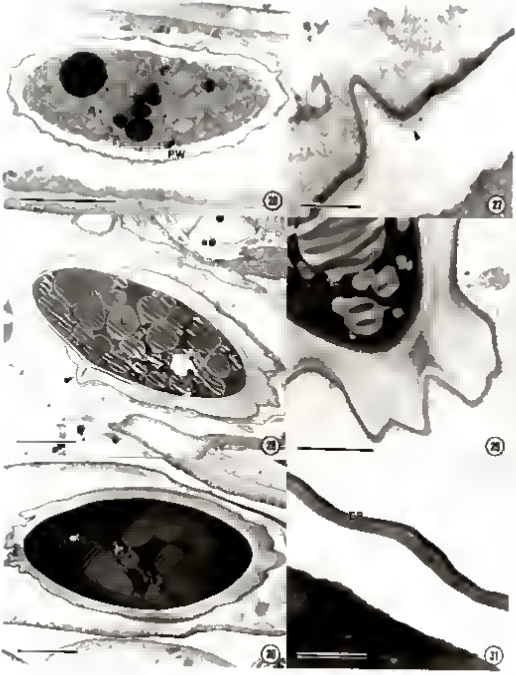
Fig. 3.27 Detailed view of the protrusion of the primary wall. A dark zone (arrow) is formed on the edge of the primary wall next to the epispore. Note the periaplasm becomes reticulate (bar = 0.5 μm).

Fig. 3.28 The primary wall becomes electron opaque and the protrusions enlarge (arrow). Numerous lipid bodies (L) are formed in the sporoplasm (bar = 5 μm).

Fig. 3.29 Detailed view of the protrusion formed by the primary wall (bar = 2 μm).

Fig. 3.30 A mature spore with osmiophilic sporoplasm (bar = 5 μm).

Fig. 3.31 A mature spore wall is composed of a primary wall with translucent and dense zones and the darker epispore (EP). The periaporic sac is collapsed (bar = 0.5 μm).


PW
27
28
29
30
31

CHAPTER IV
SPORE ONTOGENY OF *URNULA CRATERIUM*

Introduction

The genus *Urnula* was proposed by Fries (1849), who designated *Urnula craterium* (Schw.) Fr. as the type species. Anatomical studies of *U. craterium* were made by Kupfer (1902), Haald and Wolf (1910), and Wolf (1955). Later, Le Gal (1946b) studied the operculum of this genus at the light microscopic level and found that the apical chambers of *Urnula* species were either terminal and confined to the inner layer of the ascal wall or were lateral and located between inner and outer layers of the ascal wall. Because of the opercular structures, Le Gal (1946b) recognized *Urnula* as a member of the suboparculate group and later proposed Sarcoscyphaceae to accommodate this group (Le Gal, 1947). *Urnula* was included in the tribe Urnulaae which had dark colored apothecia and hymenia, in contrast to the Sarcoscyphaea which were brightly colored.

In the cytological research of Discomycetes, Barthet (1964) studied nuclear number in different tissues of Discomycetes and found that *U. craterium* had twenty-five nuclei in each ascospore. Similarly, the other *Urnula* species examined in this study had multinucleate (6-10)

ascospores. Members of Urnuleae, including U. craterium and U. pouchettii Berth. et Rious., were devoid of carotenoid pigments (Arpin, 1968). After a thorough cytochemical study of Discomycetes, Arpin came to a conclusion that the Urnuleae were derived from Sclerotiniaceae since neither contained carotenoid pigments.

Eckblad (1968) erected a new genus, Chorioactis, to include U. geaster Fuck, a species that had a geastrum-like apothecial opening. Similarly, U. platensis Speg. was transferred to Plectania Fuckel emend. Sacc. by Rifai (1968) because of apothecial characters.

Relative to pathogenicity, Urnula is a fairly unique member of the Pezizales. Most of the Pezizales are saprobic. However, U. craterium (Schw.) Fr. was determined to cause a canker disease of bur oaks (Quercus macrocarpa Mich.) (Fergus, 1951). The imperfect stage of this species is Strumella coryneoidea Sacc. & Wint. (Davidson, 1950).

Based on a unique set of characteristics, Rifai (1968) elevated Sarcoscyphaceae (sensu Le Gal) as a suborder, the Sarcoscyphineae. Later, Korf (1970) amended the definition of Sarcosomataceae proposed earlier by Kobayasi (1937) to accommodate members of the Urnuleae and included Urnula in Sarcosomateae, a tribe devoid of cyanophilous spore markings.

One aspect of ascus structure that has received the most interest in the Sarcoscyphineae has been the operculum.

At the ultrastructural level, Samuelson (1975) studied the operculum of *U. craterium* and found that the operculum, as in species of *Pseudoplectania*, was non-eccentric. He was also unable to find an incomplete ring in the ascal tip as had been depicted by Le Gal (1946a, 1946b, 1947). When comparing the ascal tips of a variety of Sarcoscyphineae, Samuelson et al. (1980) concluded that those of the Sarcosomataceae were very similar to those of the Pezizineae.

Since questions have been raised concerning the nature and presence of suboperculate asci in *Urnula* (Eckblad, 1968; Samuelson et al., 1980), the relationship of taxa in the Sarcoscyphineae becomes unclear. While the ultrastructure of spores has been examined in other Pezizales (see Chapter II), there are no data on the fine structure of spore ontogeny in species of *Urnula*. The purpose of this paper was to study ascosporogenesis of *U. craterium* ultrastructurally and to reveal information on the phylogenetic relationships of *Urnula* to the other Pezizales.

Materials and Methods

Field specimens were processed for transmission electron microscope study following the procedures described by Curry and Kimbrough (1983). The following collection was examined. *Urnula craterium* (Schw.) Fries, 5 miles N. E., Hwy 83, Forsyth Park, Macon Co., Georgia, on March 12 1992, (FLAS F55753). Ultrathin sections were obtained on an LKB

Huxley ultramicrotome with a diamond knife. After poststaining with uranyl acetate and lead citrate for 30 and 10 minutes, respectively, the sections were examined at 50 kv on a Hitachi H-600 electron microscope.

Results

Early Stage of Ascosporogenesis

The earliest stage of ascosporogenesis observed starts with the spore delimiting membrane (SDM) invaginating and encircling the eight haploid nuclei synchronously in an ascus. The apical nucleus (Fig. 4.1) and the two nuclei from the end of the ascus (Fig. 4.2) delimited by the SDM are shown. Completion of spore delimitation is later observed with a haploid nucleus enclosed by the SDM and the epiplasm becomes dense (Fig. 4.3).

Primary Wall Formation

Early primary wall development begins with primary wall material depositing between the outer and inner spore delimiting membranes (Fig. 4.4). The primary wall appears electron dense at the early stage and the mitochondria are found around the nuclear membrane (Fig. 4.5).

Formation of Perisporic Sac

After the primary wall is formed, the outer spore delimiting membrane expands around the spore to form the perisporic sac filled with electron translucent material (Fig. 4.6). The epiplasm around the elongate spore becomes condensed around the basal wall (Fig. 4.6). Later, the

perisporic sac shrinks and some vesicles appear inside the perisporic sac (Fig. 4.7). Two large vacuoles occupy most of the sporoplasm and are located on both ends of the spore (Fig. 4.7). The primary wall is electron translucent at this stage (Fig. 4.7).

Differentiation of Epispore Layers

Minute electron dense dots appear eventually on the electron translucent primary wall (Fig. 4.8). Subsequently, interactions occur between the outer spore delimiting membrane and the epiplasm occur with the appearance of some globose electron dense matrices (Fig. 4.9). The electron dense matrices seem to transfer from the epiplasm into the perisporic sac and adhere on the inner side of the outer investing membrane (Fig. 4.10). Some vesicles are left on the outer spore delimiting membrane after transfer of the electron dense matrices (Fig. 4.10). The perisporic sac is filled with loose electron dense fibrils which are partially precipitated on the primary wall and forms a distinct line on the primary wall (Fig. 4.10). At higher magnification, the distinguishing line appears as epispore precursors of radially aligned fibrils (Fig. 4.11). The granular electron dense matrix becomes spread onto the primary wall and increases the thickness of the epispore layer. As a result, the amount of the electron dense matrix decreases (Fig. 4.12).

Mitosis is observed after the epispore layer is formed. At least five nuclei are found in sections of the spore (Fig. 4.13). Simultaneously, the peripiasm is filled with electron dense granules, and electron dense bodies press onto the perisporic sac (Figs. 4.14, 4.16). Some appear to pass through the outer spore delimiting membrane (Fig. 4.17, arrow). The primary wall becomes zonated with an outer electron dense and an inner translucent zone and the epispore stains darker (Fig. 4.14). More electron dense bodies seem to transfer from the epiplasm to the perisporic sac and diffuse on the margin of the perisporic sac (Fig. 4.15). The amount of electron dense granules decreases in the perisporic sac and the epispore layer is differentiated into a dense zone sandwiched by an opaque zone and a translucent zone (Fig. 4.17).

Secondary Wall Formation

By the time the epispore layer is well differentiated, the electron opaque secondary wall material in the perisporic sac deposits on the epispore layer and the translucent zone of the primary wall expands widely (Fig. 4.17). The perisporic sac becomes fairly translucent and the sporoplasm appears osmiophilic (Fig. 4.18). Secondary wall material previously precipitated on the epispore condenses and forms a line encircling the epispore (Figs. 4.19, 4.20). Radial electron dense fibrils were observed in the encircling zone (Fig. 4.19, arrow).

The perisporic sac degenerates when the ascospore matures. Secondary wall material disintegrates and only traces of it are left (Fig. 4.20). The translucent zone of the epispore becomes more distinguishable (Fig. 4.20).

Discussion

The early stages of spore ontogeny, including spore delimitation and primary wall formation of *Urnula*, are similar to those of most Ascomycetes (Beckett, 1981). However, the entire process of ascosporogenesis of *U. craterium* may represent another type of spore wall development.

Epispore differentiation is found similar to *Hydnobolites cerebriformis* Tul. and Tul., one of the hypogeous Pezizaceae (Kimbrough et al., 1991). The similarities include the dense matrices carried by some vesicles from the epiplasm into the perisporic sac. Only the aggregation patterns of electron dense matrices show some differences between *U. craterium* and *H. cerebriformis*. In *U. craterium* the dense matrices diffuse along the inner side of the outer spore delimiting membrane but the dense bodies in *H. cerebriformis* lie on the primary wall.

Secondary wall material of *U. craterium* is also contributed by some large electron dense bodies from the epiplasm similar to those in *Ascobolus immersus* Pers. ex Pers. (Wu and Kimbrough, 1992b). Even though the initial secondary wall deposition is alike in *U. craterium* and *A.*

immersus, the mature spore wall of U. crateriumis smooth and devoid of a secondary wall layer. Ascobolus immersus, on the contrary, has a thick ornamented secondary wall.

Based upon the data obtained, U. crateriumdisplays a type of spore development with combination of the hypogeous Pezizaceae and the Ascobolaceae. However, it is very difficult to link the phylogenetic relationships among U. crateriumHydnobolitae, and Ascobolus. Morphologically, Ascobolus and Hydnobolitae both have fleshy apothecial tissues in contrast to leathery apothecial tissues of U. crateriumMost Ascobolus species are coprophilous, whereas Hydnobolites is known to form ecorrhizal associations. Urnula crateriumhowever, has been proven to be a plant pathogen that causes oak canker (Fergus, 1951). Cytologically, Berthet (1964) revealed that U. crateriumcould have up to twenty-five nuclei in each ascospore. Multinucleate ascospores are only found elsewhere in the Sarcoscyphaceae, Morchellaceae and Helvellaceae in the Pezizales. This could indicate a phylogenetic relationship of U. crateriumto these families of Pezizales.

As conserved traits, septal structures are considered to be useful in establishing phylogenetic relationships ultrastructurally (Kimbrough, 1954). The Sarcoscyphineae were found to have septal plugs more similar to those of Morchellaceae and Helvellaceae and were considered more evolved than the Pezizaceae and the Ascobolaceae according

to the complexity of septal structures. Although the septal structures of U. craterium are presented later (Chapter VII), it is not too difficult to find differences between Urnula and other members of the Pezizaceae and Ascobolaceae.

Based on ultrastructural data, I conclude that spore wall formation is not reliable in studying phylogenetic relationships of the Pezizales. Different families may have a very similar type of ascospore development, and different species of the same genus may present different patterns of spore wall deposition (Chapter II). Urnula craterium is merely another example that shows the variations of spore wall development. More information may be needed to determine phylogenetic relationships of Urnula to the other families of the Pezizales.

Figs. 4.1-4.9 Transmission electron micrographs of spore wall ontogeny in Urnula craterium.

Fig. 4.1 The top nucleus (N) in the ascus is delimited by spore delimiting membrane (SDM) (bar = 2 μm).

Fig. 4.2 The two nuclei from the base of the same ascus are delimited by spore delimiting membrane (bar = 2 μm)

Fig. 4.3 Completion of spore delimitation. A haploid nucleus (N) is encircled by spore delimiting membrane (bar = 2 μm).

Fig. 4.4 Primary wall (PW) deposition begins between the inner and the outer spore delimiting membranes (arrows) (bar = 2 μm).

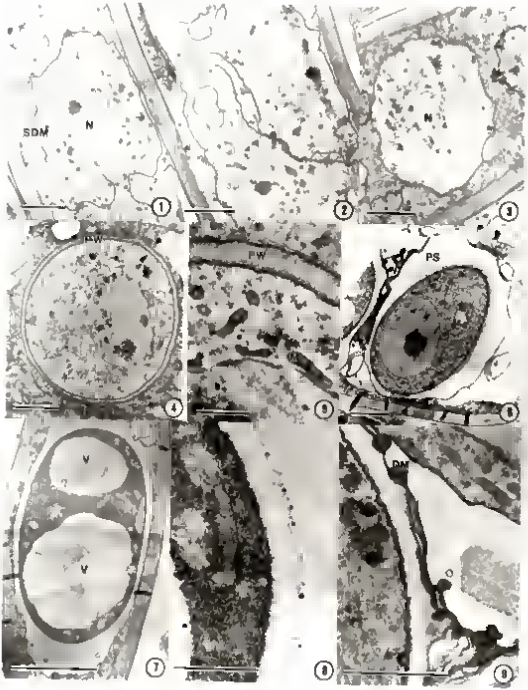
Fig. 4.5 Detailed view of primary wall (PW) deposition. The primary wall appears slightly electron dense. Note some mitochondria (M) are around the nuclear membrane (bar = 0.5 μm).

Fig. 4.6 The outer spore delimiting membrane expands and forms the perisporic sac (PS) around the spore (bar = 2 μm).

Fig. 4.7 The perisporic sac reduced on one end of the spore. Two large vacuoles (V) appear in the sporoplasm (bar = 4 μm).

Fig. 4.8 Some small electron dense dots deposit on the primary wall (bar = 0.5 μm).

Fig. 4.9 Electron dense matrices (DM) within the spiplasm and the perisporic sac appear to interact with the outer spore delimiting membrane (bar = 1 μm).


SDM
N
N
1
2
3
PW
PW
PS
4
5
6
V
V
DM
7
8
9

Figs. 4.10-4.17 Transmission electron micrographs of spore wall ontogeny in Urnula craterium.

Fig. 4.10 The electron dense matrices (arrows) diffuse on the inner side of the outer spore delimiting membrane. Some electron dense material precipitates on the primary wall and forms the episporo precursors (EPP). Note the vesicles left on the spore delimiting membrane (bar = 0.5 μm).

Fig. 4.11 Detailed view of the episporo precursors (EPP) and undulation of the sporoplasma membrane (arrow) (bar = 0.5 μm).

Fig. 4.12 Pronounced precipitation of episporo precursors on the primary wall. Small amount of electron dense matrices are still attached on the inner side of the outer spore delimiting membrane (bar = 0.5 μm).

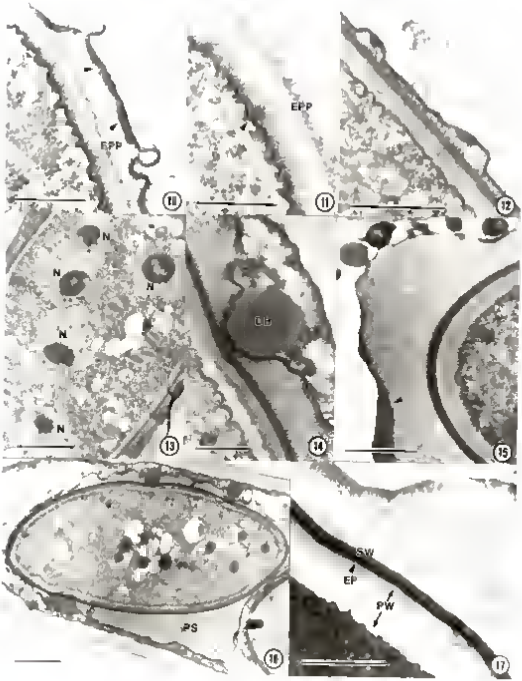
Fig. 4.13 Mitosis occurs in the ascospore and five nuclei (N) are observed in the section (bar = 2 μm)

Fig. 4.14 Detailed view of the electron dense body (DB) appearing to penetrate the perisporic sac (bar = 0.5 μm).

Fig. 4.15 More electron dense bodies seem to transfer from the epiplasm to the perisporic sac and diffuse on the margin (arrow) of the perisporic sac (bar = 1 μm)

Fig. 4.16 The perisporic sac (PS) is filled with electron dense granules. Some electron dense bodies press on and appear to penetrate into the perisporic sac (bar = 2 μm)

Fig. 4.17 The wall differentiated ascospore (EP) is composed of a dense zone, a translucent zone and an opaque zone. A layer of secondary wall (SW) material deposits on the ascospore. Electron dense material in the perisporic sac becomes loose and the primary wall (PW) is zonated (bar = 0.5 μm).

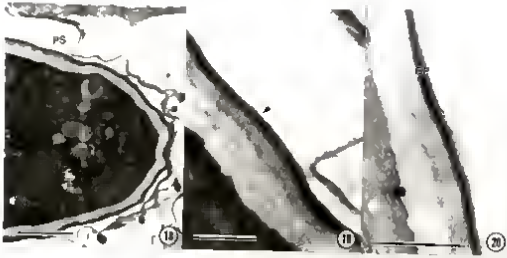

EPP
EPP
N
N
N
N
N
DE
SW
EP
PW
PS
10
11
12
13
14
15
16
17

Figs. 4.18–4.20 Transmission electron micrographs of spore wall ontogeny in *Urnula craterium*.

Fig. 4.18 The periasporic sac (PS) becomes electron translucent and the sporoplasm is osmiophilic (bar = 2 μm).

Fig. 4.19 Part of the secondary wall material disintegrates and some radial fibrils are left on the epispore (arrow). Note the distinguishing line between secondary wall material and the epispore (bar = 0.5 μm)

Fig. 4.20 Mature spore wall is devoid of secondary wall material and the translucent zone in the epispore (EP) becomes more distinguishable (bar = 0.5 μm).


PS
19
18
20

CHAPTER V
SPORE ONTOGENY OF *PSEUDOPLECTANIA NIGRELLA* AND *PLECTANIA NANNFELDTII*

Introduction

Pseudoplectania and *Plectania* were both erected by Fuckel (1870), and their respective type species are *P. nigrella* (Pers. ex Fr.) Fckl. and *P. melastoma* (Sow. ex Gray) Fuckl. These two genera are similar in having blackish discoid-shaped apothecia. They differ in that *Pseudoplectania* has globose spores and *Plectania* ovoid to ellipsoid ones. Boudier (1885) recognized another genus, *Melascypha*, to include *M. melaena* (Fr.) Boud., which was referred to as a synonym of *Pseudoplectania* by Seaver (1928). Seaver (1942) later studied *Pseudoplectania* and *Plectania* morphologically. He provided a key and descriptions of *Pseudoplectania* species, including *P. nigrella*. However, he interpreted *Plectania* to include the genus *Sarcoscypha*, which has brightly colored apothecia and hymenia. This misinterpretation was followed by some mycologists (Korouse, 1948; Nannfeldt, 1949; Le Gal, 1953, Berthet, 1964). At the same time, Seaver placed some *Plectania* species under *Bulgaria*. Not only was *Plectania* misinterpreted as *Sarcoscypha*, but it was merged with *Urnula* (Le Gal, 1946b, 1958a; Berthet, 1964). Because *Urnula* had

larger apothecia than Plectania and was lacking a thick gelatinous layer, several mycologists suggested that Urnula and Plectania should be kept separated (Nannfeldt, 1949, Korf, 1957; Dennis, 1960; Moser, 1963; Eckblad, 1968).

Aside from taxonomical and morphological studies, Le Gal (1946b) found that the operculer structures of Discomycetes were useful in studying phylogenetic relationships among Discomycetes at the light microscopic level. She also concluded that there was a group of Discomycetes that had open rings on the top of the asci (suboperculs), including P. melastoma, Ps. nigrella, and Pa. vogesiaca (Pers.) Sacc. (= M. melaena). Thus, the Sarcoscyphaceae was proposed to accommodate suboperculate members (Le Gal, 1947) and was separated into two tribes, the Sarcoscypheae and the Urnuleae. Pseudoplectania and Plectania were arranged in the Urnuleae and the species studied had a terminal chamber located inside the inner layer of the sacal wall (Le Gal, 1946b).

Plectania has been mistakenly shifted among genera because of variability of its spore shape and by the presence or absence of transverse furrows on the spores. Based upon spore characters, Korf (1957) seperated Plectania into three sections, Plectania, Curvatisporae, and Plicosporae, and treated the other generic names as synonyms. P. nannfeldtii Korf was placed in the section Plectania.

Several years later, Berthet (1964) studied the cytology of Discomycetes and found that Ps. nigrella had two to four nuclei in each spore, whereas, P. melastoma, and P. platensis (Speg.) Rifai had six to ten, and eight to ten nuclei, respectively. He also found that the entire Sarcoscyphaceae (sensu Le Gal) was characterized by multinucleate spores. Cytochemical studies done by Arpin (1968) revealed that Pseudoplectania and the other Urnuleae were devoid of carotenoid pigments. Arpin also suggested that the Urnuleae were derived from the Sclerotiniaceae. Since the Sarcoscyphaceae had a number of unique characteristics, Rifai (1968) created a suborder, Sarcoscyphineae, to distinguish Sarcoscyphaceae from the other Pezizaies. In the same study, Rifai provided descriptions of four Plectania species, and at least three of them had cyanophobic horizontal ridges on the ascospores. He did not study species of Pseudoplectania.

Although Le Gal's (1947) taxonomic scheme was followed until 1970s, the family name Sarcosomataceae, proposed by Kobayasi (1937) for certain suboperculate Discomycetes, was overlooked. Korf (1970) followed Rifai (1968) in recognizing the Sarcoscyphineae but seended the Sarcosomataceae and included two families in the suborder, Sarcoscyphaceae and Sarcosomataceae, to represent the Sarcoscyphaes and Urnuleae, respectively. Korf (1970, 1973) used the staining reaction of spore markings to divide the

Sarcosomataceae into two tribes, the Sarcosometeae without cyanophilic spore markings and the Galielleae with cyanophilic ornaments. Both Pseudoplectania and Plectania were included in the Sarcosomataeae. At the ultrastructural level, Samuelson (1975) and Samuelson et al. (1980) examined operculer structures of the suboperculate group, including Pa. nigrella, and found that the opercular structures of the Sarcosometaceae were non-eccentric and similar to those of Pezizinaee. Beliemere et al. (1990) also studied the operculs and ascal wall of members of Sarcosomataceae, including Pa. nigrella and P. melastoma, and concluded that the Sarcosomataceae were not suboperculate. A general view of the ascospore wall was shown in the same study (Beliemere et al., 1990).

Only very limited ultrastructural studies have been done on species of Pseudoplectania and Plectania and most of these were restricted to the ascal wall. The cyanophobic spore markings used in distinguishing Sarcosomateae and Galielleae still need to be examined at the ultrastructural level in order to compare the cyanophobic ridges found in the Sarcoscyphaceae (Chapter III). The origin of the spore markings can only be found by means of ultrastructural study (personal observations with the light microscope). The purpose of this paper is to study spore wall ontogeny by transmission electron microscopy and hopefully resolve these problems and provide information on the phylogenetic

relationship of Pseudoplectania and Plectania to the other Sarcosomataceae and Pezizales

Materials and Methods

Field specimens were processed for transmission electron microscope study following the procedures described by Curry and Kimbrough (1983). The following collections were examined: Pseudoplectania nigrella (Pers. ex Fr.) Fuckel, on moist, mossy soil near snow melt, 5000 ft. elevation, on Timberline Lodge Rd., 300 yds off Hwy 26, Mt. Hood, 2 mi. E. of Government Camp, Oregon, on June 24 1984, (FLAS F53942); Plectania nannfeldtii Korf, on conifer twigs along snowbank, 1/4 mi. behind Campground at Yuba Pass, 6800 ft. elevation, 5 mi. E. of Sierra City, Sierra Co., California, on June 20 1984, (FLAS F53955). Ultrathin sections were obtained on an LKB Huxley ultramicrotome with a diamond knife. After postaining with uranyl acetate and lead citrate for 30 and 10 minutes, respectively, the sections were examined at 50 kv on a Hitachi H-600 electron microscope.

Results

Early Stage of Ascosporogenesis

The earliest stage of ascosporogenesis observed in Pe. nigrella starts with spore delimiting membrane (SDM) encircling a haploid nucleus and associated cytoplasm and organelles (Fig. 5.1). The spore delimiting membrane is composed of two layers of double unit membranes (Fig. 5.2).

The same stage of ascosporogenesis was not observed in the *P. nannfeldtii*.

Primary Wall and Perisporic Sac Formation

The primary wall (PW) material is deposited between the outer and inner spore delimiting membranes and is electron translucent in both *Pa. nigrella* (Fig. 5.3) and *P. nannfeldtii* (Fig. 5.5). At the same stage, some electron dense dots appear outside the primary wall in *Pa. nigrella* (Fig. 5.3, arrows). Higher magnification shows that the outer spore delimiting membrane slightly expands and forms the perisporic sac (PS) and the electron dense dots are attached to the outer spore delimiting membrane (Fig. 5.4). The epiplasm (EP) appears somewhat electron dense in *Pa. nigrella* (Figs. 5.3, 5.4) and is filled with loose electron dense granules in *P. nannfeldtii* (Fig. 5.5).

Differentiation of Epispore Layers

Subsequently, the perisporic sac is filled with electron dense fragmented matrices (FM) in *Pa. nigrella* (Fig. 5.6) and some of the matrices seem to be deposited on the primary wall and form the epispore precursors (EPP) composed of radial fibrils (Fig. 5.7). At this stage, the primary wall is no longer electron translucent (Figs. 5.6, 5.7) and the sporoplasm becomes more vacuolate (Fig. 5.6). Another discrete electron dense matrix (DM) also appears in the perisporic sac (Fig. 5.8) and contributes to the epispore precursors (Fig. 5.9, arrow).

The amount of epispore precursors increases (Fig. 5.10) and the perisporic sac (PS) further expands and is filled with electron dense granules (Fig. 5.11). Simultaneously, the epispore (EP) becomes zonate (Fig. 5.11) and an uneven staining reaction occurs in the primary wall (Figs. 5.11, 5.12, arrows). After the epispore is formed, the perisporic sac becomes more electron dense, especially on its margin (Fig. 5.12). Large electron dense globular bodies (GB) appear to be engulfed by the outer spore delimiting membrane and part of the dense material in the perisporic sac becomes deposited on the epispore (Fig. 5.13, arrow). Some vesicles (VS) appear in the epiplasm at the same stage (Fig. 5.13). The large dense bodies are dispersed on the outer spore delimiting membrane (Fig. 5.14). Later, the electron dense secondary wall material (SWM) in the perisporic sac becomes homogeneous and the epiplasm still has some large electron dense matrix (Fig. 5.15).

In P. nannfeldtii, the process of epispore differentiation was not observed. However, the epispore appears to be a single electron dense layer with small dense dots (arrows), and the periplasm (PSM) outside of the epispore is filled with electron dense secondary wall material (Fig. 5.16). The primary wall is differentiated into two zones, the narrow inner band and the outer unevenly thick zone (Fig. 5.16). The wall differentiated epispore layer in Pa. nigrella is composed of one dark intermediate

layer, one lighter inner layer and one lighter outer layer (Fig. 5.17)

Secondary Wall Formation

After spispore formation is completed in Ps. nigrella, the periplasm becomes less dense and the perisporic sac reduces in size (Fig. 5.18). in P. nannfeldtii, the dense dots formed by secondary wall material on the spispore layer increase in size. The perisporic sac is reduced considerably with only the outer spore delimiting membrane attached to the dense dots (Fig. 5.19, arrows). it is similar in Ps. nigrella except that there are no electron dense dots (Fig. 5.20). The primary wall also becomes electron translucent at this stage (Fig. 5.19). in contrast to P. nannfeldtii, the primary wall in Ps. nigrella is slightly electron opaque encircled by an electron dense line (Fig. 5.20, arrow).

Mature Spore Wall Formation

The sporoplasm in the mature spore of P. nannfeldtii is electron dense with osmiophilic lipid bodies (L) arranged around inside the primary wall. The outer spore wall is undulate (Fig. 5.21). The mature spore of Ps. nigrella also has osmiophilic sporoplasm (Fig. 5.22) and an undulate spore wall (Figs. 5.22, 5.23).

With higher magnification, the mature spore wall of Ps. nigrella is composed of the primary wall and the spispore without any secondary wall (Fig. 5.23). in P. nannfeldtii, the mature spore wall is composed of the primary wall, the

ascospore with two lighter and two dark alternating layers, and small ornamenta (OR) formed by secondary wall material (Fig. 5.24).

Discussion

The entire process of spore wall development found in *Ps. nigrella* is similar to *Sphaerosporella* in the Otideaceae (Wu, 1991). Both of the genera have electron dense secondary wall material accumulating in the perisporic sac during ascospore and primary wall development. *Pseudoplectania nigrella* and *Sphaerosporella* are also similar in that both lack a secondary wall layer. However, there are still some differences between *Ps. nigrella* and *Sphaerosporella*. First, the ascospore of *Ps. nigrella* is composed of four layers while that in *Sphaerosporella* is multi-layered (more than four layers). *Sphaerosporella* also has an outer banded primary wall while *Ps. nigrella* has a homogeneous electron translucent primary wall.

Even though the similarities of the ascosporogenesis between *Ps. nigrella* and *Sphaerosporella* might indicate a certain relationship, stronger evidence for proving such a relationship is still needed. From a view of the habitats, *Ps. nigrella* is usually associated with decaying coniferous wood, or on *Sphagnum* (Seaver, 1942), while *Sphaerosporella* is on burned substrates. The specificity existing between the fungus and the wood may suggest a host-pathogen relationship. On this point, *Ps. nigrella* shares a common

feature with *Ornula cretorum*, another member of the Sarcoscomataceae which causes oak canker. In contrast to *Sphaeroeporella*, the characteristic of multinucleate ascospores of *Ps. nigrella* found by Berthet (1964), although a multinucleate spore was not observed in this study, indicates that *Ps. nigrella* may be genetically more closely related to multinucleate taxa such as Morchellaceae and Helvellaceae.

Many of the Sarcoscyphineae have more or less synchronous spore development and do not produce apothecia in culture. Only a few stages of spore development in *P. nannfeldtii* were found in field collections. Based on the key provided by Korf (1957), *P. nannfeldtii* has smooth ascospores. However, at the ultrastructural level, the ascospore has secondary wall ornaments. In the study of *Phillipsia* and *Wynnea* (Chapter III), the spore markings composed of primary wall material are cyanophobic. On the contrary, species with secondary wall spore ornaments are cyanophilic. Therefore, the ultrastructural evidence presented here suggests that spore ornaments found in *P. nannfeldtii* should stain blue in cotton blue if the staining reaction of the spore markings are visible under a light microscope. I rehydrated two herbarium specimens of *P. malastoma* (CUF, MM-1743; FLAS F53940) with distilled water at 4°C overnight, stained them with lactophenol cotton blue, and examined the specimens under a light microscope at high

magnification (X2000). I was not abia to find tha cyanophobic ridges as described by Rifai (1968). On the contrary, a thin layer of secondary wall staining blue by cotton blue was found in both specimens. Korf (personal communication) also found that P. campyiospora (Berk.) Nannf. apud Korf had cyanophilic spora markings. Taxonomically, Korf (1970, 1973) placed P. nannfeldtii and Pe nigrella in the Sarcosomateae, a triba of Sarcosomataceae which ara devoid of cyanophilic spore markings. Since Pa. nigrella lacks a secondary wall layer which stains blue in cotton blue, thare is no problem to retain this species in the Sarcosomataae. However, species of Plectania may not be abia to fit into this scheme.

Tha method of ascosporogenesis may not provide enough information to show phylogenetic relationships among Sarcosomataceae and tha other Pezizales. However, tha composition of spora markings may be useful for taxonomic study. Ultrastructural and light microscopic data show that so far, the cyanophobic spore markings formed by primary wall material may exist in the Sarcoscyphaceae and not in tha Sarcosomatoceae. Because of tha discovery of tha secondary wall ornaments in P. nannfeldtii, other members of tha Sarcosomataae should be examined to determine if cyanophilic secondary walls or spora markings characterize tha family.

Figs. 5.1-5.9 Transmission electron micrographs of spore wall ontogeny in *Pseudoplectania nigrella* and *Plectania nannfeldtii*.

Fig. 5.1 Early ascosporogenesis shows the spore delimiting membrane (SDM) encircling a haploid nucleus (N) in *Ps. nigrella* (bar = 2 μm).

Fig. 5.2 The spore delimiting membrane of *Ps. nigrella* is composed of two layers of double unit membranes (bar = 0.5 μm).

Fig. 5.3 Electron translucent primary wall (PW) material deposited between the inner and outer spore delimiting membranes in *Ps. nigrella*. Note some electron dense dots appear outside of the primary wall (arrows) (bar = 2 μm).

Fig. 5.4 Higher magnification shows that the electron dense dots are attached on the expanding outer spore delimiting membrane (SDM) in *Ps. nigrella* (bar = 0.5 μm).

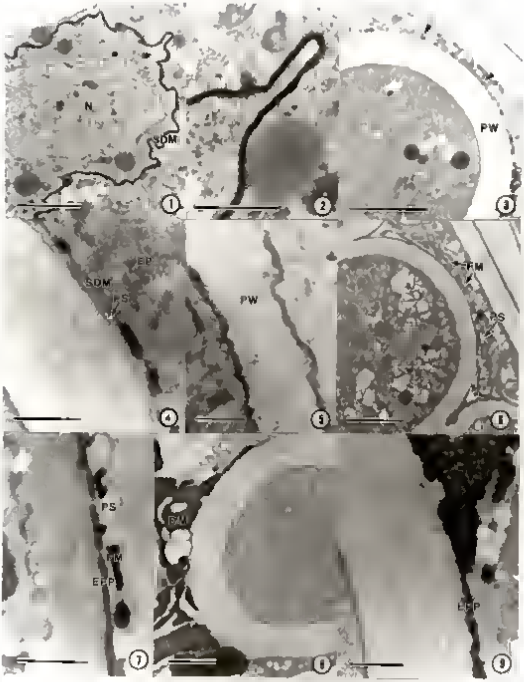
Fig. 5.5 Electron translucent primary wall (PW) material deposited between the inner and outer spore delimiting membranes in *P. nannfeldtii* (bar = 0.5 μm).

Fig. 5.6 The perisporic sac (PS) is filled with electron dense fragmented matrices (FM) in *Ps. nigrella* (bar = 2 μm).

Fig. 5.7 The electron dense fragmented matrices (FM) in the perisporic sac (PS) in *Ps. nigrella* seem to deposit on the primary wall and form the epispore precursors (EPP) (bar = 0.5 μm).

Fig. 5.8 Other electron dense discrete matrices (DM) appear later in the perisporic sac in *Ps. nigrella* (bar = 1 μm).

Fig. 5.9 Some of the dense discrete matrices are deposited on the primary wall as epispore precursors (EPP) in *Ps. nigrella* (bar = 0.5 μm).


N
SDM
1
2
PW
3
EP
SDM
PS
4
PW
5
FM
PS
6
PS
FM
EPP
7
BM
8
EPP
9

Figs 5.10-5.18 Transmission electron micrographs of spore wall ontogeny in *Pseudoplectania nigrella* and *Plectania nannfeldtii*.

Fig. 5.10 The amount of the epispore precursors (EPP) increases in *Ps. nigrella* (bar = 0.25 μm).

Fig. 5.11 The perisporic sac (PS) further expands and is filled with electron dense granules and the epispore (EP) becomes zonated in *Ps. nigrella*. Note the different staining reaction (arrows) in the primary wall (PW) (bar = 0.5 μm).

Fig. 5.12 The margin of the perisporic sac becomes more electron dense in *Ps. nigrella* (bar = 0.5 μm)

Fig. 5.13 A large electron dense globular body (GB) seems to be engulfed by the outer spore delimiting membrane in *Ps. nigrella*. Note some electron dense material is deposited on the epispore (arrow) (bar = 0.5 μm)

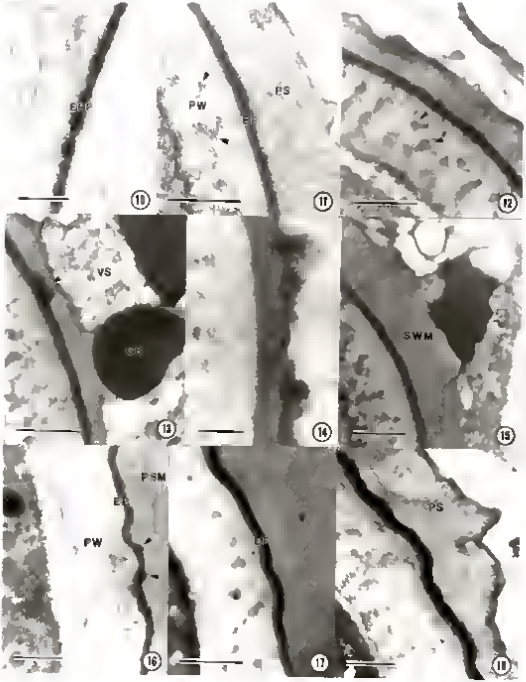
Fig. 5.14 The large dense body seems to spread and interact with the outer spore delimiting membrane in *Ps. nigrella* (bar = 0.25 μm).

Fig. 5.15 The secondary wall material (SWM) in the perisporic sac appears homogeneous and the epiplasm still has some large electron dense bodies in *Ps. nigrella* (bar = 0.5 μm).

Fig. 5.16 Some electron dense dots (arrows) are attached on the single-layered epispore (EP) of *P. nannfeldtii*. The primary wall (PW) is differentiated into two zones and the periplasm (PSM) is filled with secondary wall material (bar = 0.5 μm).

Fig. 5.17 The well differentiated epispore (EP) layer in *Ps. nigrella* is composed of one dark intermediate layer and lighter outer and inner layers (bar = 0.5 μm)

Fig. 5.18 The perisporic sac (PS) of *Ps. nigrella* reduces after the epispore is fully differentiated and the periplasm becomes less electron dense (bar = 0.25 μm)


PW
PS
VS
SWM
PSM
PW
PS
10
11
12
13
14
15
16
17
18

Figs. 5.19-5.24 Transmission electron micrographs of spore wall ontogeny in *Pseudopisectania nigrella* and *Plectania nannfeldtii*.

Fig. 5.19 Secondary wall material deposited on the epispore as electron dense dots (arrows) increases in size and the perisporic sac collapses with the outer spore delimiting membrane (SDM) attaching on the secondary wall in *P. nannfeldtii* (bar = 0.5 μm).

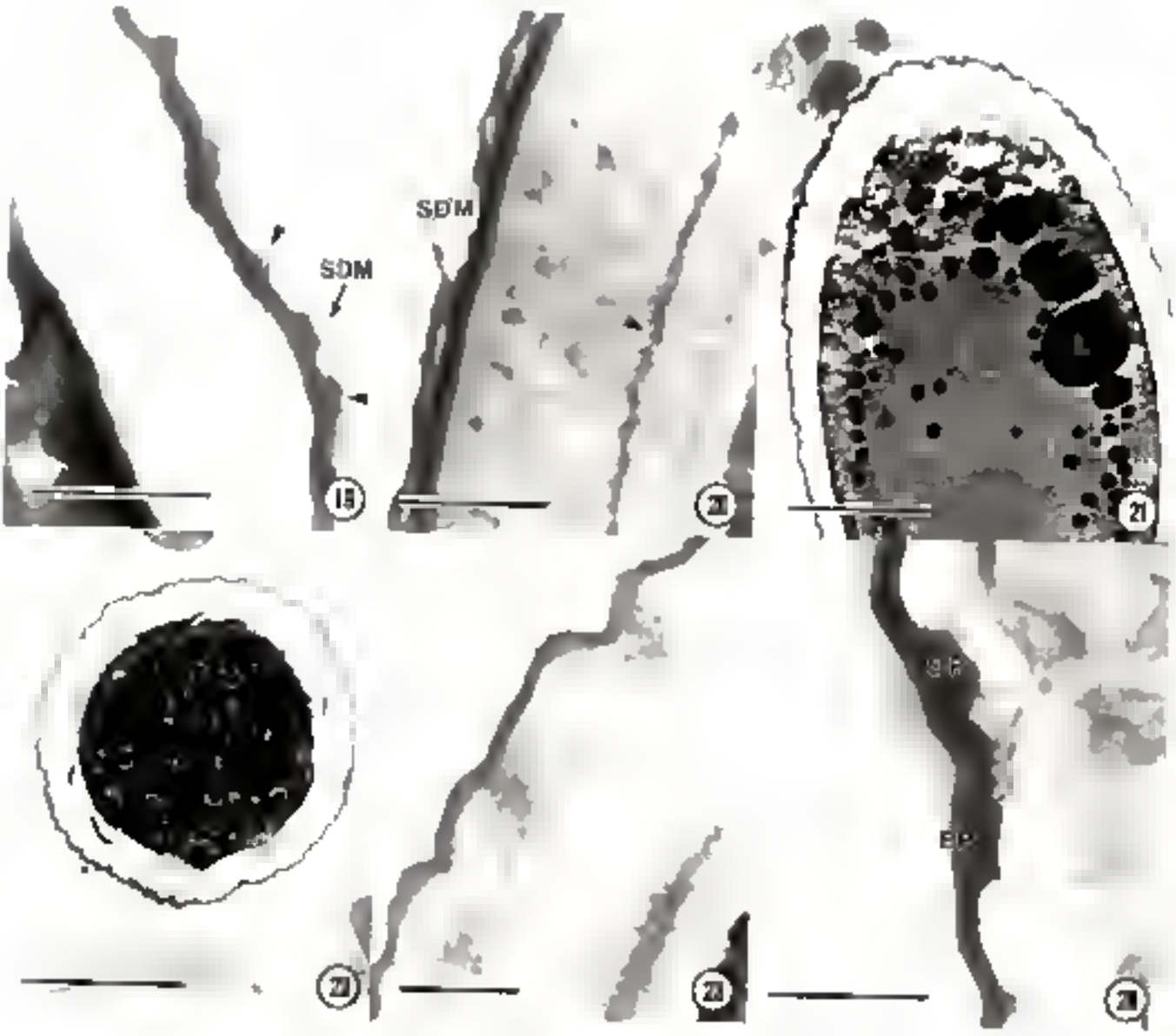
Fig. 5.20 A similar stage in *Ps. nigrella* with spore delimiting membrane (SDM) covering the epispore. An electron dense line (arrow) appears in the primary wall (bar = 4 μm).

Fig. 5.21 The mature spore *P. nannfeldtii* is ellipsoid with an undulate spore wall. Several osmophilic lipid bodies (L) are in the sporoplasm (bar = 4 μm).

Fig. 5.22 The mature spore of *Ps. nigrella* is globose and the spore wall is undulate (bar = 5 μm).

Fig. 5.23 Detailed view of mature spore wall in *Ps. nigrella*. Note the epispore is exposed without any secondary wall on the outside (bar = 0.5 μm).

Fig. 5.24 Detailed view of mature spore wall in *P. nannfeldtii*. The fully differentiated epispore (EP) is composed of two electron dense and two lighter alternating layers. Ornaments (OR) formed by secondary wall material adhere on the epispore (bar = 0.5 μm).


SDM
SDM
18
20
21
22
23
24

CHAPTER VI
SPORE ONTOGENY OF GALIELLA RUFA

Introduction

Galiella rufa (Schw.) Nannf. & Korf was first described as Bulgaria rufa Schweinitz (1832) and transferred to Sarcosoma rufum (Schw.) Rehm (1886) Seaver (1928) and Le Gal (1953), however, recognized the name B. rufa. Later, Kobayasi (1937) examined Bulgaria and Sarcosoma species and concluded that Bulgaria should belong to the inoperculate group and Sarcosoma to the operculate group. Because of the gelatinous apothecia of Sarcosoma, he proposed a new family, Sarcosomataceae, to include the genera that had gelatinous apothecia. In the same study, S. platydiscus (Cesp.) Sacc. var. celebicum (Henn.) Kobayasi, which is now considered a species of Galiella, was included in Sarcosoma. This is the first time that a species we consider in Galiella was placed in the suboperculate group. Unfortunately, the family name Sarcosomataceae was overlooked and Le Gal (1947) proposed another name, Sarcoscyphaceae, to represent the group with suboperculate asci

Nannfeldt and Korf (Korf, 1957) found that some Sarcosoma species recognized by Le Gal (1953) had cyanophilous ornamented spores which were different from the

smooth spores of Sarcosoma. Therefore, Gallalla was proposed as a genus in the Sarcoscyphaceae to accommodate the species with cyanophilous spore markings. Galialla was first introduced with four species G. javanica (Rehm in P. Hauu.) Nannf. & Korf, G. thwaitesii (Berk. & Br.) Nannf., G. celebica (P. Henn. in Warb.) Nannf., and the type species, G. rufa (Korf, 1957). Korf (1957) also suggested that Galialla may be more evolved than Plectania, another genus in the Sarcoscyphaceae (sensu Le Gal), by having lighter colored apothecia and a more pronounced gelatinous layer.

Taxonomically, Eckblad (1968) placed Gallalla in Urnuleae, one of the tribes of Sarcoscyphaceae based upon the dark colored apothecia. Based on their unique characteristics, Rifai (1968), who did not study Gallalla species, proposed a new suborder in the Pezizales, the Sarcoscyphineae, which included the members of Sarcoscyphaceae. Two years later, Korf (1970) divided Sarcoscyphineae into two families, the Sarcoscyphaceae with brightly colored apothecia, and the Sarcosomataceae with dark-colored apothecia. Galialla was placed in Sarcosomataceae, tribe Galiellaea, with cyanophilous spore markings. Unlike the other Sarcoscyphineae, the data on carotenoid pigments and unclear number in G. rufa are lacking.

Most studies of G. rufa are so far confined to morphological features and a few ultrastructural works on opercula and the ascal wall. Ultrastructurally, Samuelson et al. (1980) found that the opercula of Sarcosomatacaae, including G. rufa, were non-eccentric and not different from those of the other Pezizales except the Sarcoscyphaceae. Bellamère et al. (1990) also concluded that the Sarcosomataceae were not suboperculate. However, these workers considered Galiella neither as a member of the Sarcoscyphaceae nor of the Sarcosomataceae because the subdivision of the apical ascal wall layers was different from that of the other Sarcosomataceae.

A number of questions have been raised relative to the position of the Sarcosomataceae in the suborder Sarcoscyphineae. Little is known about the nature of ascosporogenesis in members of this suborder. In previous studies the ultrastructure of spore ontogeny has been determined in species of Sarcoscypha (Chapter II), Phillipsia and Wynnea (Chapter III), Urnula (Chapter IV), and Pseudoplectania and Plectania (Chapter V). The purpose of this paper is to describe ultrastructural techniques used to study ascosporogenesis in Galiella rufa and reveal cytological information that can be used to establish phylogenetic relationships to the Sarcoscyphaceae and other families of Pezizales.

Materials and Methods

Field specimens were processed for transmission electron microscope study following the procedures described by Curry and Kimbrough (1983). The following collection was examined. Galiella rufa (Schw.) Nannf. & Korf; on decaying wood found under oaks, Botanical Garden of University of Georgia, Athens, Clarke Co., Georgia, on July 11 1992, collected by J. W. Kimbrough and Li-Tzu Li, (FLAS F55949). Ultrathin sections were obtained on an LKB Huxley ultramicrotome with a diamond knife. After poststaining with uranyl acetate and lead citrate for 30 and 10 minutes, respectively, the sections were examined at 50 kv on a Hitachi H-600 electron microscope

Results

Early Stage of Ascosporogenesis

Prior to ascosporogenesis, the ascus contains a diploid nucleus (N) and different sizes of vesicles (V) with electron dense matrices located above and below the nucleus (Fig. 6.2). The events of meiosis, mitosis, and ascus vesicle membrane delimitation were not observed. The completion of spore delimitation is shown with a single haploid nucleus completely encircled by spore delimiting membranes (SDM) and some primary wall (PW) material deposited between the outer and the inner spore delimiting membranes (Fig. 6.3).

Primary Wall and Perisporic Sac Formation

During primary wall formation, eight ascospores in one ascus develop synchronously (Fig. 6.1). More primary wall material is deposited between two layers of the spore delimiting membrane. The primary wall (PW) is electron translucent, and the ascospores are globose (Fig. 6.4). At this stage, only two nuclei are found in the upper spore, and numerous electron dense globular bodies (GB) appear in the epiplasm (EPM) (Fig. 6.4). These globular bodies appear to be present in electron translucent vesicles (TV) scattered throughout the epiplasm (Figs. 6.4-6.7).

Later, these large vesicles with inclusions become appressed to the outer spore delimiting membrane (Fig. 6.5). Most of the material in the vesicle is electron translucent but with many electron dense globular bodies which become amorphous and surrounded with electron dense granules (Fig. 6.5).

In the next stage of wall development, the outer spore delimiting membrane expands at various places along the primary wall (Fig. 6.6, arrow). After the perisporic sac (PS) is formed, the spore elongates and becomes more or less ovoid, and electron dense bodies are found in the sporoplasm (Fig. 6.6). At higher magnification, the globular bodies in the epiplasmic vesicles change in electron density, attach to the outer spore delimiting membrane, and seem to spread (arrow) into the perisporic sac (Fig. 6.7).

Differentiation of Epispore Layers

Subsequently, two types of aggregation occur in the perisporic sac, one in which small, globose electron dense bodies (arrows) attach to the primary wall, and the other in which large, astral matrices appear close to the inner side of the outer spore delimiting membrane (Figs. 6.8, 6.9). Epispore precursors (EPP) develop between the globular bodies on the primary wall (Fig. 6.9). At this stage, electron dense granules appear in the primary wall (Figs. 6.8, 6.9). Certain areas of the ascospore wall are covered by radially arranged epispore precursors. The outer spore delimiting membrane is pressed very close to the epispore precursors by the epiplasm, which has mitochondria (M) (Fig. 6.10). The primary wall appears more electron dense and slightly zonated (Fig. 6.10).

By the completion of epispore formation, the ascospore is narrowly elliptical, and the organelles in the sporoplasm are seen clearly, including a couple of nuclei (Fig. 6.11). After the first layer of the epispore (EP) is formed, the perisporic sac is filled with electron dense granules and other amorphous electron opaque bodies (OB) (Fig. 6.12). These bodies adhere to the first layer of the epispore, and large discrete masses (DM) deposit on them (Fig. 6.13). The opaque bodies later appear firmly attached to the epispore layers, and the large discrete masses enlarge and become more compact in density (Fig. 6.14). The wall

differentiated epispore is composed of multiple layers and the electron translucent primary wall has radial striations in the outer area (Fig. 6.14).

Mature Spore Wall

The large discrete masses later disappear from the perisporic sac and the perisporic sac completely disintegrates (Figs. 6.15, 6.16) with only traces of the outer spore delimiting membrane left (Fig. 6.16, arrow). The sporoplasm becomes osmiophilic in the mature spore, and only lipid bodies (L) can be easily distinguished within (Fig. 6.15). The mature spore wall is composed of a zonate primary wall, an epispore, and a secondary wall which has opaque bodies as ornaments (OB) (Figs. 6.15, 6.16).

Discussion

The early stages of ascosporogenesis found in G. rufa are the same as those in most of the Ascomycetes (Beckett, 1981). However, the primary spore wall of G. rufa is different from Phillipsia and Wynnea (Chapter III) by lack of the primary wall ornaments. In general, the entire process of spore wall development of G. rufa is similar to the other Sarcoscomataceae regardless of the presence of globular electron dense bodies in the epiplasm before epispore layer differentiation. Also, G. rufa and Plectania nannfeldtii Korf (Chapter V) both have fine ornaments composed of secondary wall material. Since ascospores of G. rufa and P. nannfeldtii are both ellipsoid and ornamented,

it is very difficult to distinguish those two simply by comparing spore wall formation.

Other than the other members in the Sarcosomataceae, secondporogenesis of G. rufa is similar to Discina, the subgenus of Gyromitra, in Helvellaceae (Kimbrough and Wu, 1990), especially the electron dense globular bodies found outside of the periporic sac which are engulfed later by the outer spore delimiting membrane. The differences between Gyromitra and G. rufa are that G. rufa has amorphous globular electron dense bodies before epispore formation, two different types of wall material aggregation during epispore layer differentiation, and secondary wall formation that differs from species of Gyromitra.

The cyanophilous ornaments of G. rufa were found to be composed of secondary wall material. This discovery supports my earlier observations that the secondary wall stains blue in cotton blue dyes. Taxonomically, with the cyanophilous spore markings and the fairly extensive gelatinous layer in the apothecium together with other unique features found in the Sarcosomataceae, G. rufa represents a typical member of the Sarcosomataceae, tribe Galiellaceae. It may not be necessary, however, to pull Galiella out of the Sarcoscyphineae based only upon subdivisions of the operculer wall layers (Bellemère et al., 1990).

Cytological data show that G. rufa has multinucleate ascospores (Figs. 6-4, 6.11), which is one of the features that characterize the Sarcoscyphineae as well as the Helvellaceae and the Morchellaceae. Gallella rufa may be more closely related to the Morchellaceae and the Helvellaceae, including the discinoid Gyromitra, based on this genetic characteristic.

So far, studies of ascosporogenesis at the ultrastructural level can only provide limited information for helping to solve some of the taxonomic problems. Even though G. rufa is possibly closely related to other groups with multinucleate spores, more concrete evidence, such as septal structures which are considered to be the most conservative trait, will be needed to show phylogenetic relationships of G. rufa to other Pezizales and the direction of evolution in the Sarcosomataceae.

Figs 6.1-6.7 Transmission electron micrographs of spore wall ontogeny in *Galleria rufa*.

Fig. 6.1 An ascus (AS) with eight haploid ascospores developing more or less synchronously. The paraphyses (P) arranged parallel to the ascus (bar = 5 μm)

Fig. 6.2 An ascus with a diploid nucleus (N). Note the different sized vesicles (V) carrying electron dense material below and above the nucleus (bar = 4 μm).

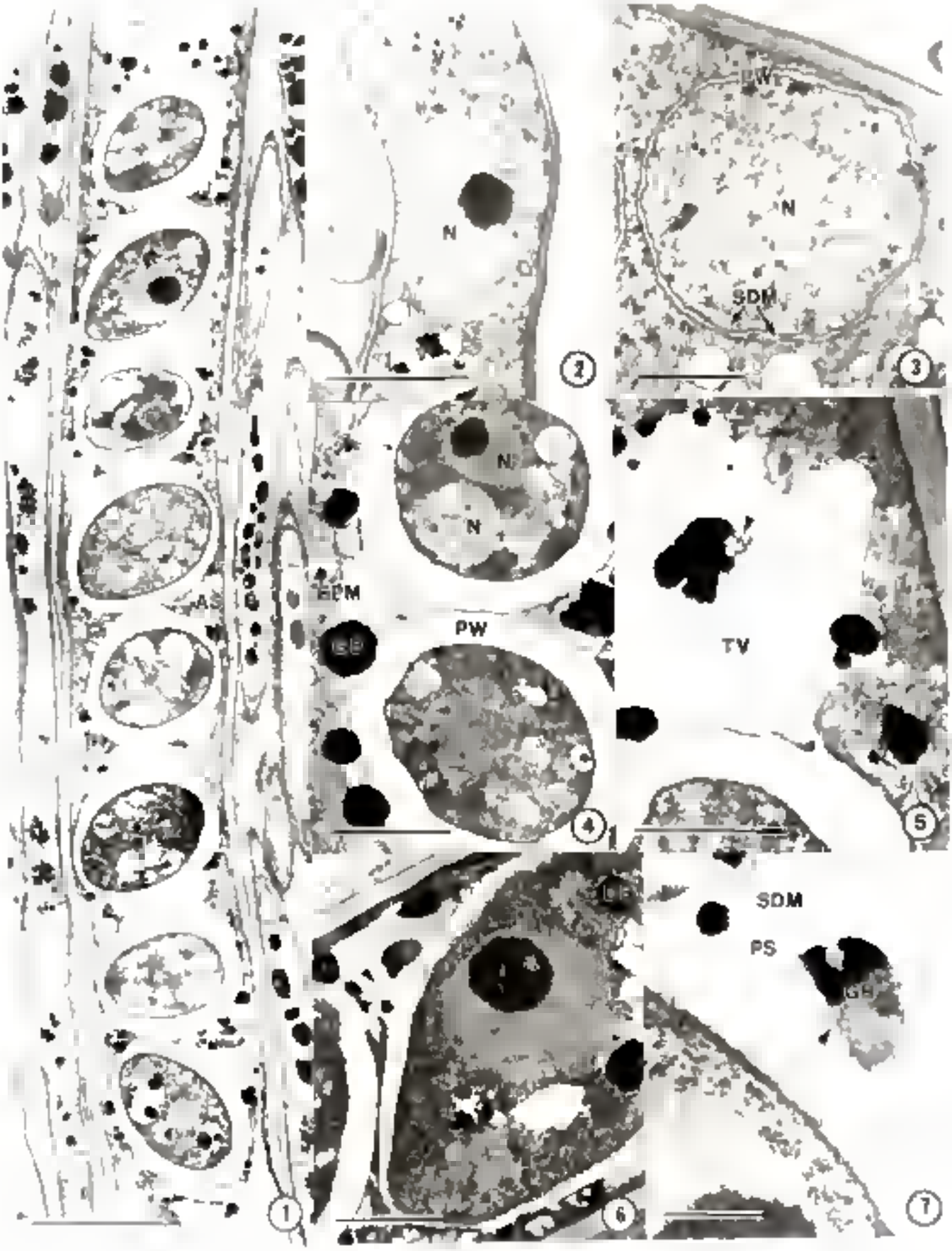
Fig. 6.3 The ascospore has a haploid nucleus (N) after spore delimitation and the primary wall (PW) material begins to deposit between the outer and the inner spore delimiting membranes (SDM) (bar = 2 μm)

Fig. 6.4 Two adjacent spores with the transincent primary wall (PW) and electron dense globular bodies (GB) in the epiplasm (EPM). Note the upper spore is multinucleate (N) (bar = 3 μm).

Fig. 6.5 The electron transincent vesicles (TV) containing globular bodies appress on the outer spore delimiting membrane. The globular bodies become amorphous and surrounded by electron dense granules (arrow) (bar = 2 μm)

Fig. 6.6 The ascospore becomes more or less ovoid with several dense bodies (DB) in the sporoplasm. The outer spore delimiting membrane expands at various places along the primary wall (arrow) (bar = 2 μm)

Fig. 6.7 The globular bodies (GB) change in electron density, attach to the outer spore delimiting membrane (SDM), and appear to spread (arrow) into the perisporic sac (PS) (bar = 0.5 μm)


N
SDM
PW
N
N
SDM
PW
GB
TV
SDM
PS
GB
1
2
3
4
5
6
7

Figs 6.8 6.15 Transmission electron micrographs of spore wall ontogeny in *Galiella rufa*.

Fig. 6.8 Two types of electron dense material aggregates in the perisporic sac, one in which small, globose electron dense bodies (arrows) attach on the primary wall (PW), and the other where large, astral matrices appear close to the inner side of the outer spore delimiting membrane (SDM) (bar = 1 μm).

Fig. 6.9 Detailed view of the two types of electron dense material aggregating in the perisporic sac. Epispore precursors (EPP) develop between the small electron dense bodies and the primary wall has electron dense granules (bar = 0.5 μm).

Fig. 6.10 The radially arranged epispore precursors (EPP) adhere to the slightly zonated primary wall and are very close to the outer spore delimiting membrane (SDM). Note the mitochondria (M) in the epiplasm (bar = 0.5 μm).

Fig. 6.11 The multinucleate (N), ellipsoid spore with organelles seen clearly (bar = 2 μm).

Fig. 6.12 Amorphous electron opaque bodies (OB) are formed by secondary wall material in the perisporic sac and some of them are slightly attached on the first layer of epispore (EP). Note the primary wall is more electron dense (bar = 0.5 μm).

Fig. 6.13 Large discrete masses (DM) appear in the perisporic sac and cover the opaque bodies (bar = 1 μm).

Fig. 6.14 Large discrete masses become homogeneous and more compact in density. The amorphous opaque bodies are firmly attached onto the multilayered epispore (EP). Note the translucent primary wall with striations in the outer area (bar = 0.5 μm).

Fig. 6.15 The sporoplasm of a mature spore is osmiophilic and contains many lipid bodies (L). Note the outermost layer of spore wall is ornamented and the perisporic sac is disintegrated (bar = 2 μm).

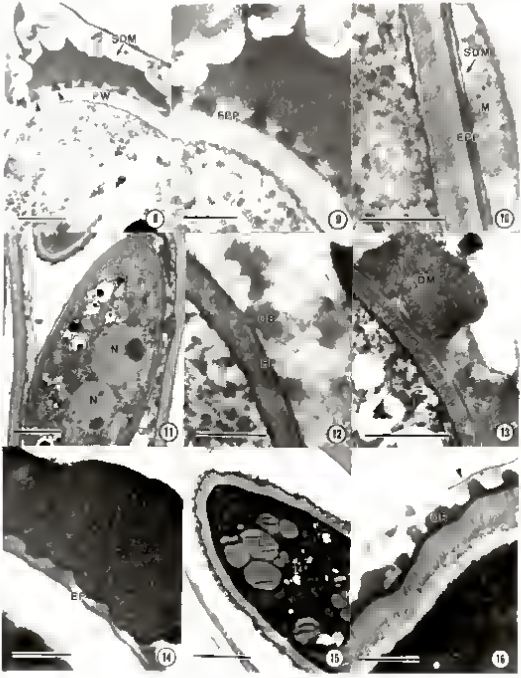

SDM
PW
8
EBP
9
SDM
M
EBP
10
N
N
11
GB
EP
12
DM
13
EP
14
15
GB
16

Fig. 6_16 The mature spore wall is composed of the zonated primary wall, the epispore, and the ornaments (OR) originating from secondary wall material. Traces of the outer spore delimiting membrane (arrow) is left after the periporic sac degenerated (bar = 0.5 μm)

CHAPTER VII
SEPTAL STRUCTURES IN THE SARCOSCYPHACEAE AND SARCOSOMATACEAE

Introduction

The Sarcoscyphaceae and Sarcosomataceae belonging to the suborder Sarcoscyphineae in the Pezizales are characterized by a series of unique features, including leathery apothecia, an aporhynque type of crozier system, and especially an obliquely positioned operculum (Rifai, 1968). Since Chadefaud (1945) and Le Gal (1946a, 1946b) described the suboperculum and the apical open ring of the asci, studies of members of this suborder have been limited mostly to morphology (Le Gal, 1947, Eckblad, 1968; Rifai, 1968, Korf, 1970, 1973), cytology (Berthet, 1964), and cytochemistry (Arpin, 1968).

Several phylogenetic schemes were suggested for the Sarcoscyphaceae and Sarcosomataceae. Based upon the opercular structures that were found in the suboperculate asci, Le Gal (1946b) hypothesized that the operculate Discomycetes were derived from the inoperculate Discomycetes by way of the suboperculate group. Her scheme was adopted by Korf (1958), Berthet (1966), and Arpin (1968). Berthet (1966) hypothesized that, through parallel evolution, the suboperculate and the operculate groups were derived from

the Sclerotiniaceae and Geoglossaceae, respectively. The inoperculate Discomycetes and the Sarcoscyphineae represented the end points of evolution in their respective groups. Arpin (1968) agreed with Berthet (1966) and further suggested that the Sclerotiniaceae was the ancestor of the Sarcosomataceae, whereas the origin of the Sarcoscyphaceae was unknown. Eckblad (1968) considered that the proposal of a relationship between the suboperculate and the inoperculate group was weak due to his being unable to find the apical open ring as described by Chadefaud (1946) and Le Gal (1946a, 1946b) in fresh materials. Eckblad felt that the apical open ring was an artifact of fixation and suggested that the Sarcoscyphineae were derived from operculate Discomycetes and could be a final step in the evolution in the Pezizales. However, because the Sarcoscyphineae had a number of unique characteristics, Eckblad (1968) even suggested that the recent ancestors of the Sarcoscyphineae were probably extinct. Other characteristics are needed, however, to confirm whether this evolutionary scheme is valid.

Early history of ultrastructural studies of septal structures was summarized by Curry and Kimbrough (1983), and subsequent studies of septa in families of the Pezizales showed that septal structures in different tissues of various families can be used in helping to solve phylogenetic and taxonomic problems. Carroll (1967)

demonstrated a complicated type of septal plugs in Ascodesmis. Later, Kamaletdinova and Vasilyev (1983) studied septal structures of several families in the Pezizales, including the Sarcoscyphaceae and showed that both pore plugs and Woronin bodies were variable in shape. Curry and Kimbrough (1983) studied Pezizaceae and found that the septal structures in ascal bases and ascogenous hyphae consisted of either convex or biconvex bands, whereas those in the vegetative hyphae were laminated structures associated with globose Woronin bodies around the pore. In the Ascobolaceae (Kimbrough and Curry, 1985), similar hemispherical pore plugging structures were found in both ascal bases and ascogenous hyphae, and electron opaque bands with sometimes indistinct laminations were found in the somatic hyphae. However, Curry and Kimbrough (1983) found in Iodophanus an almost identical pattern of septal structures to those in the Pezizaceae. Therefore, they suggested that Iodophanus should be considered as a member of the Pezizaceae. Sclerotia in the Otideaceae had different types of septal structures in excl of Octospora, Aleuria, Anthracobia, and Pulvinula (Kimbrough and Curry, 1986a; Wu, 1991). Sowerbyellaceae, Scutellinieae, Lachneae, some of the Aleurieae in the Otideaceae, and Ascobolaceae shared similar types of septal structures (Kimbrough and Curry, 1985, 1986a, 1986b, Wu, 1991). Members of the Helvellaceae were found to have a unique cone-shaped

electron opaque body associated with V-shaped striations in the septal pore of sacl (Kimbrough and Gibson, 1989) Similar type of pore plugging structure was later discovered in Gaopyxis of the Otidiaceae (Kimbrough and Gibson, 1990) A phylogenetic scheme based upon septal structures of the Pezizales was proposed by Kimbrough (1994).

Ultrastructural studies of the Sarcoscyphaceae and Sarcosomataceae are restricted to operculo and secal walls (Samuelson, 1975; Samuelson et al., 1980; Donadini et al., 1989; Beliemere et al., 1990; Maiéndez-Howell et al., 1990). The data demonstrated that opercula of the Sarcoscyphaceae were eccentric and were different from the other Pezizales, including the Sarcosomataceae of which opercula were non-eccentric. My work on spore wall ontogeny of the selected genera in the Sarcoscyphaceae and Sarcosomataceae (Chapter II; Chapter III; Chapter IV; Chapter V; Chapter VI) showed a great deal of variation. Due to these inconsistencies, spore wall ontogeny may not be reliable in studying phylogenetic relationships in the Pezizales. However, the origin of the Sarcoscyphaceae and Sarcosomataceae and the real affinity between these two families has been discussed, but no one has been able to reach a conclusion (Le Gal, 1946b; Berthet, 1966; Arpin, 1968; Eckblad, 1968). Since the septal structures of other Pezizales were found to be useful in resolving taxonomic problems at the family level and to show phylogenetic relationships within the Pezizales

(Kimbrough, 1994), septal structures associated with sici, secondary hyphae, and vegetative hyphae were investigated. The purpose of this paper is to describe the results of these studies and to show relationships of the Sarcoscyphaceae and Sarcosomataceae to the Pezizales based on septal structures.

Materials and Methods

Field specimens were processed for transmission electron microscope study following the procedures described by Curry and Kimbrough (1983). The following collections were examined. Sarcoscypha occidentalis (Schw.) Sacc.; off Bullpen Road, 4.5 mi. S. E. of Highlands, Macon Co., North Carolina, on August 4 1987, (FLAS F55272); Sarcoscypha coccinea (Jacq. : Fr.) Lambotte, on decaying hardwood, Watkins Mill, 5 mi. E. of Kearny, Davies Co., Missouri, on April 26 1992, (FLAS F55878); Phillipsia domingensis (Berk.) Berk., S. W. 20th Avenue, near US 75, Gainesville, Alachua Co., Florida, on September 16 1983, (FLAS F53645); Wynnea americana Berk. & Curt., Boil Creek Road, Hydrology Research area, Cowasta, Macon Co., North Carolina, on August 14 1987, (FLAS F55286), on August 14 1992, (FLAS F55394); Urnula craterium (Schw.) Fries; Hwy 83, 5 mi. N. E., Forsyth Park, Macon Co., Georgia, on March 12 1992, (FLAS F55753); Pseudoplectania nigrella (Pers. ex Fr.) Fckl., on moist, mossy soil near snow melt, 5000 ft elevation, on Timberline Lodge Road, 300 yds. off Hwy 26, Mt. Hood, 2 mi. E. of

Government Camp, Oregon, on June 24 1984, (FLAS F53942), Plectania nannfeldtii Korf; on conifer twigs along snowbank, 1/4 mi. behind campground at Yuba Pass, 6800 ft, 5 mi. E. of Sierra City, Sierra Co., California, on June 20 1984, (FLAS F53955); Galiella rufa (Schw.) Nannf. & Korf, on decaying wood found under oaks, Botanical Garden of University of Georgia, Athens, Clarke Co., Georgia, on July 11 1992, (FLAS F55949). Ultrathin sections were obtained on an LKB Huxley ultramicrotome with a diamond knife. After poststaining with uranyl acetate and lead citrate for 30 and 10 minutes, respectively, the sections were examined at 50 kv on a Hitachi H-600 electron microscope.

Results

Septal Structures in the Sarcoscyphaceae

Excipulum and paraphyses. Excipular septa of S. occidentalis are uniperforate. Septal structures include an electron dense band loosely attached to the pore rim and several globose Woronin bodies (W) in the adjacent cytoplasm (Fig. 7.1). In P. domingensis (Fig. 7.2) and W. americana (Fig. 7.3), uniperforate septa of the excipular cells are plugged by an electron opaque fan-shaped matrix surrounded by a number of globose electron dense Woronin bodies. Both of the septal plugs in P. domingensis and W. americana appear homogeneous with a translucent torus on each side of the plugs (arrows) (Figs. 7.2, 7.3).

The pore plug in paraphyses of S. occidentalis is either an electron opaque fan-shaped matrix lightly attached to the septum (Fig. 7.4) or two small electron opaque matrices that adhere to both sides of the septum (Fig. 7.5). In W. americana, the septal structures are composed of an electron dense matrix with laminations bordering both sides of the septum (Fig. 7.6, arrows), and are entrapped by the overgrown secondary wall (SW).

Ascogenous hyphae. In young apothecia of S. coccinea, ascogenous hyphae with trailing hyphae were found. At the distal end toward the ascus mother cell (ASM), septal structures (arrow) are observed at the septum between the ascogenous hypha (AH) and the ascus mother cell with a dikaryotic nucleus (N) (Fig. 7.7). At higher magnification, the central pore of the septum is plugged by a poorly differentiated electron opaque matrix and surrounded by several microbody-like vesicles (V) with electron dense material inside (Fig. 7.8). The septal plug in P. domingensis is a pulley-shaped electron opaque matrix with a few electron opaque bodies around it (Fig. 7.9). No Woronin bodies were found in the ascogenous hyphae of either S. coccinea or P. domingensis. However, microbody-like vesicles were found near the pores in S. coccinea.

Asci. The septa of the ascal base were found only in S. occidentalis. The ascal base septum is uniperforate and the septal structure develops as a fan-shaped electron

opaque matrix at both sides of the septum (Fig. 7.10). However, the fan shaped matrix at the side of the nacus (AS) is not well differentiated and is associated with an electron translucent zone (arrows) on each side of the matrix (Fig. 7.10).

Septal Structures in the Sarcosomataceae

Excipulum and paraphyses. The excipular septal structures in U. craterium are composed of a hemispherical electron dense matrix and globose Woronin bodies (Fig. 7.11). In addition to globose Woronin bodies, short cylindrical, hexagonal (Fig. 7.12), and long cylindrical (Fig. 7.13) Woronin bodies were also found in excipular cells.

In G. rufa, the septal structure of the excipular cell is a pulley-shaped electron dense matrix with indistinct striations and an electron translucent border on each side of the matrix (Fig. 7.14, arrows). Woronin bodies associated with this type of septal plug are elliptical (Fig. 7.14) or cylindrical (Fig. 7.15). Some pore plugs in G. rufa have slightly opaque bands with a darker line (arrow) running across the plug and along the septum (Fig. 7.16). Woronin bodies accompanying this type of septal structure are more or less globose (Fig. 7.16). The septal structures in the excipular cells of P. nannfeldtii are two electron dense matrices attached to both sides of the septum and surrounded by globose Woronin bodies (Fig. 7.17).

The pore plug of paraphyses in U. craterlum is hemispherical and electron dense with globose Woronin bodies appressed to it (Fig. 7.18). A fan-shaped electron opaque matrix and globose Woronin bodies are observed in the paraphyses of Pa. nigrella (Fig. 7.19). In paraphyses of P. nannfeldtii, an electron dense matrix is trapped within the overgrown secondary wall, but globose Woronin bodies remain outside of the secondary wall (Fig. 7.20).

Secogenous hyphae. In the members of the Sarcosomataceae examined, septal structures of secogenous hyphae were observed only in U. craterlum. The plug of the central uniperforate septum is composed of a dumbbell-shaped electron opaque matrix with indistinct laminations and a translucent border on both sides of the septum (Fig. 7.21).

Asci. In U. craterlum, the central pore plug of the basal base septum is a dumbbell-shaped matrix with V-shaped bands (arrows) and a translucent border on each side of the septum (Fig. 7.22). The V-shaped bands are better differentiated on the secogenous hyphal side (SH) of the basal base than those of the secus side (SS) of the septum (Fig. 7.22).

A similar dumbbell shaped electron opaque matrix is found in G. rufa (Fig. 7.23). Two electron dense striations (arrows) appear in both sides of the dumbbell-shaped matrix. A translucent torus separates the matrix from both sides of the septum (Fig. 7.23). The pore plug of Pa. nigrella is an

electron dense matrix with slightly electron opaque material in the center (Fig. 7.24). However, the entire structure is trapped with an overgrowth of the secondary wall and is difficult to see in detail (Fig. 7.24).

Discussion

Because the Sarcoscyphineae have the unique feature of an aporhynque type of crozier, i.e. trailing hyphae, even with well-oriented material, it becomes very difficult to obtain septal structures of ascal bases or in recognizing the exact cell types present. Since Woronin bodies are only found in excipular cells, paraphyses, and occasionally in ascogenous hyphae, but not in ascal bases, this character is used to distinguish vegetative and reproductive hyphae.

In members of the Sarcoscyphaceae examined, the septal structures found in the vegetative hyphae are of two different types. Except for the laminated structures of paraphyses in *W. americana*, they are typical of most of the families in Pezizales (Branner and Carroll, 1968; Curry and Kimbrough, 1983; Kamaletdinova and Vasaliyev, 1983; Kimbrough and Curry, 1986a, 1986b; Kimbrough and Gibson, 1989, Wu, 1991) and *Hydnobolites*, a truffle genus found linked to the Pezizaceae (Kimbrough et al., 1991). Other septal structures in *S. occidentalis*, *P. domingensis*, and in excipular cells of *W. americana*, are similar to those found in some of the Otideaceae (Kimbrough and Curry, 1986a, Kimbrough and Gibson, 1990, Wu, 1991) and Ascobolaceae

(Kimbrough and Curry, 1985). Septal pore plugs associated with ascogenous hyphae of *S. coccinea*, and *P. domingensis*, and especially those of ascal bases in *S. occidentalis*, are almost identical to those in *Mycolachnea*, *Trichophaea* (Wu, 1991), and *Genea*, also a truffle genus related to *Mycolachnea* (Otideaceae) (Li and Kimbrough, 1994). The microbody-like vesicle found in *S. coccinea* is very similar to the peculiar microbodies found by Wergin (1973) in a species of *Fusarium*.

The septal pore structures found in the Sarcosomataceae display various patterns. *Urnula craterium* has identical pore plugs in both excipular cells and paraphyses, similar to those in *S. occidentalis* and *P. domingensis*. However, the hexagonal Woronin bodies in excipular cells of *U. craterium* are also found in *Verpa* (Morchellaceae) (Kamaletdinova and Vasiliyev, 1983), *Anthracobia*, *Octospora* (Alauriaceae, Otideaceae) (Kimbrough and Curry, 1986a), and *Calycypha* (Sowerbyellaceae, Otideaceae) (Kimbrough and Curry, 1986b). The long cylindrical Woronin bodies in *U. craterium* are only found elsewhere in *Morchella*, *Verpa* (both Morchellaceae) (Kamaletdinova and Vasiliyev, 1983), and in species of *Gyromitra* (Helvellaceae) (Kimbrough, 1991).

The septal structures of ascogenous hyphae and of ascal bases in *U. craterium* are almost identical to those of *Geopyxis* (Otideaceae). The septal structures of vegetative hyphae in *G. rufa* display two unique patterns in which in

one there is a small granular matrix and in the other a prominent pore plug with a translucent torus. This torus is less distinct or missing in members of the Sarcoscyphaceae examined. Similar to U. craterium, long cylindrical Woronin bodies are also observed in the excipular cells. The pore plugging structure of the ascal base in G. rufa is more or less similar to that of Caloscypha of the Otideaceae (Kimbrough and Curry, 1986b). Septal pore organelles associated with vegetative hyphae in P. nannfeldtii and Pa. nigrella, and those in S. occidentalis, P. domingensis, W. americana are alike in appearance.

In either vegetative or reproductive hyphae of both the Sarcoscyphaceae and Sarcosomataceae, septal pore plugs sometimes are trapped in the overgrown secondary wall. Similar observations were made in different families of the Pezizales (Curry and Kimbrough, 1983, Kimbrough and Curry, 1985, 1986a, 1986b, Kimbrough, 1991).

Septal structures of ascal bases in P. domingensis, W. americana, and P. nannfeldtii were not found. In general, the septal structures found in ascal bases and ascogenous hyphae indicate that the Sarcoscyphaceae are related to both the Lachnea (Otideaceae) and the Sarcosomataceae. They are also intermediate between the Alaurieae (Otideaceae) and the Morchellaceae (see Chapter VIII). Likewise, based upon the fan shaped, laminated types of septal structures in vegetative hyphae in the Sarcoscyphaceae and the

Sarcosomataceae, these two families are related to each other and to the Otideaceae of the Pezizales.

Both Sarcoscyphaceae and Sarcosomataceae possibly have a polyphyletic origin because of the variation of septal structures in both vegetative and reproductive hyphae. In the Sarcoscyphaceae, despite the lack of septal data in oscogenous hyphae and basal bases of W. americana, the septal plug of paraphyses indicates that W. americana could be different from Sarcoscypha and Phillipsia. Wynnea also has clustered apothecia which are more complicated than the solitary discoid apothecia of Sarcoscypha and Phillipsia. However, Phillipsia and Wynnea both have cyanophobic spore markings originating from the primary wall, while Sarcoscypha has only smooth spores (Chapter II, III). Different types of septal structures in basal bases are also found in the Sarcosomataceae.

Berthet (1964) found multinucleate spores in the Sarcoscyphaceae and Sarcosomataceae and thought that multinucleate groups were more advanced than the Pezizales with uninucleate spores (Berthet, 1966). From the data that I obtained, the Sarcoscyphaceae and Sarcosomataceae appear more closely related to the multinucleate groups, the Morchellaceae and Helvellaceae. They are possibly derived from the Otideaceae, a uninucleate group, especially Geopyxis and members of the Aleuria-Otidea complex. These data also indicate that the Sarcoscyphaceae and

Sarcoscoetaceae are farther removed from the Pezizaceae which have the simplest septal structures in the Pezizales. Therefore, the phylogenetic scheme for the Sarcoscyphineae proposed by Le Gal (1946b), in which the suboperculate group was derived from the inoperculate group, may not stand since, based on septal structures, the Pezizaceae appear to be at the base of the phylogenetic tree. I agree with Eckblad (1968) that the Sarcoscyphineae are derived from certain members of the Pezizales, and that the phylogenetic relationships using septal structures proposed by Kimbrough (1954) appear reasonable.

Figs. 7.1-7.5 Transmission electron micrographs of septal structures in taxa of Sarcoscyphineae.

Fig. 7.1 Sarcoscypha occidentalis with an electron dense band (arrow) in the central pore of the septum (S) surrounded by several globose Woronin bodies (W) in the excipular cells (bar = 1 μm).

Fig. 7.2 Phillipsia domingensis with a fan-shaped matrix and electron translucent torus (arrows) plugging the unperforate septum. Several globose Woronin bodies in the excipular cells (bar = 0.5 μm).

Fig. 7.3 Wynnea americana with a similar fan shaped septal plug and electron translucent torus (arrows) and globose Woronin bodies in the excipular cells (bar = 0.5 μm).

Fig. 7.4 Sarcoscypha occidentalis with a fan-shaped electron opaque matrix in the septal pore of the paraphysis and globose Woronin bodies (bar = 0.5 μm).

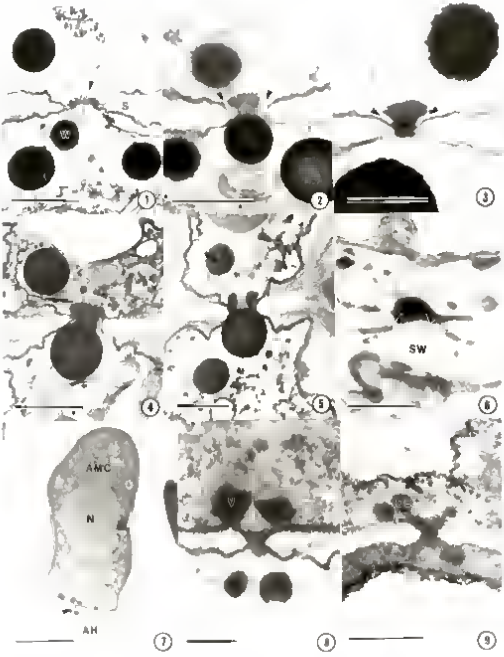
Fig. 7.5 Sarcoscypha occidentalis with two small matrices attached to both sides of the paraphysis septum. Note a Woronin body plugs the central pore (bar = 2 μm).

Fig. 7.6 Wynnea americana with a laminated (arrows) matrix attached to both sides of the paraphysis septum by electron dense borders. Note the laminated matrix is trapped in the secondary wall (SW) (bar = 0.5 μm).

Fig. 7.7 An ascogenous hyphe (AH) with a pore plug (arrow) in the septum separating the ascus mother cell (AMC) in Sarcoscypha coccinea. Note the large diploid nucleus (N) in the ascus mother cell (bar = 2 μm).

Fig. 7.8 Detailed view of the septal structure of the ascogenous hyphe in Sarcoscypha coccinea. An electron opaque matrix in the central pore associated with several microbody-like vesicles (V) carrying electron dense material (bar = 0.25 μm).

Fig. 7.9 Phillipsia domingensis with a pulley-shaped electron opaque matrix and a few electron opaque bodies (OB) in the ascogenous hyphe (bar = 0.5 μm).


W
1
2
3
4
5
SW
6
AMC
N
AH
7
8
9

Figs. 7.10-7.18 Transmission electron micrographs of septal structures in taxa of Sarcoscyphineae

Fig. 7.10 A fan-shaped pore plugging structure of the ascal base with two electron translucent zones (arrows) on the side of the ascus (AS) in Sarcoscypha occidentalis (bar = 0.5 μm)

Fig. 7.11 Urnula craterium with a hemispherical septal plug with globose Woronin bodies in the excipular cell (bar = 0.25 μm).

Fig. 7.12 Urnula craterium with short cylindrical and hexagonal Woronin bodies (W) in the excipular cell (bar = 0.5 μm).

Fig. 7.13 Urnula craterium with electron dense long cylindrical Woronin bodies in the excipular cell (bar = 0.5 μm).

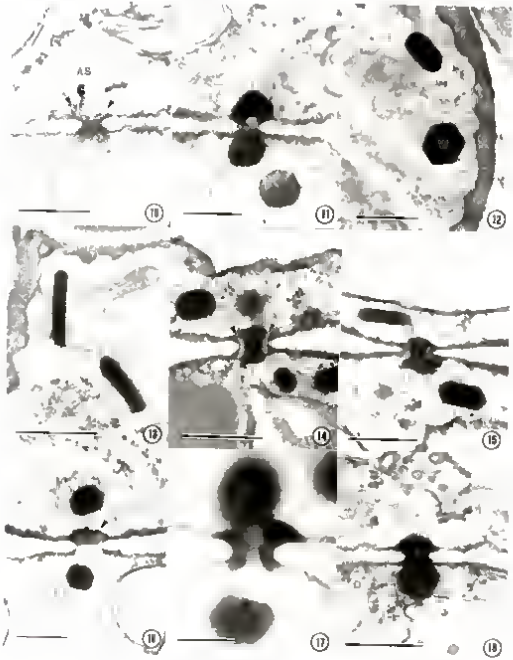
Fig. 7.14 A septal structure showing a pulley-shaped matrix with indistinct striations and two translucent borders (arrows) on both sides in the excipular cell of Galiella rufa. Note the Woronin bodies are more or less elliptical (bar = 0.5 μm).

Fig. 7.15 Galiella rufa with long cylindrical Woronin bodies around the septal pore of the excipular cell (bar = 0.5 μm).

Fig. 7.16 Another type of septal structure of the excipular cell in Galiella rufa showing a slightly electron opaque band with a darker line (arrow) crossing the septum and the band (bar = 0.25 μm).

Fig. 7.17 Plectania pannfeldtii with two electron dense matrices adhering on both sides of the septum and a few globose Woronin bodies in the excipular cell (bar = 0.25 μm).

Fig. 7.18 Urnula craterium with a hemispherical pore plug attached to a globose Woronin body in the paraphysis (bar = 0.5 μm).


AS
10
11
12
13
14
15
16
17
18

Figs. 7.19-7.24 Transmission electron micrographs of septal structures in taxa of Sarcoscyphinae.

Fig. 7.19 Pseudoplectania nigrella with a fan-shaped electron opaque matrix in the central pore surrounded by several globose Woronin bodies (W) in the parephysis (bar = 0.25 μm).

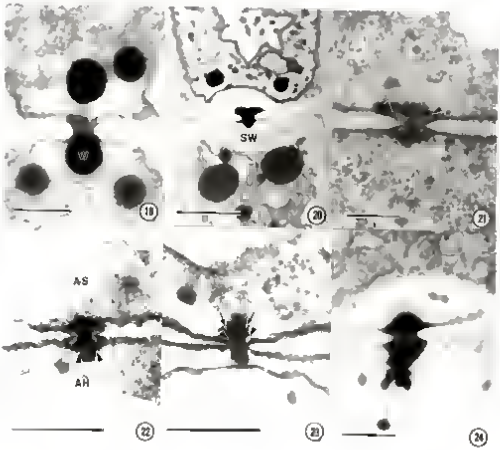
Fig. 7.20 Plectania nannfeldtii with an electron dense matrix in the septal pore in the paraphysis. The matrix is trapped in the secondary wall (SW) and a few globose Woronin bodies surround the septum (bar = 1 μm).

Fig. 7.21 The secogenous hypha of Urnula craterium showing a dumbbell-shaped matrix with striations and electron translucent borders (arrows) on both sides of the septum (bar = 0.25 μm).

Fig. 7.22 A pore plug in the ascal base of Urnula craterium showing a dumbbell-shaped matrix with V-shaped striations (arrows) more prominent in the secogenous hyphal (AH) than the ascal (AS) sides of the septum and electron translucent borders (bar = 0.5 μm).

Fig. 7.23 A septal structure in the ascal base of Galiella rufa showing a dumbbell-shaped matrix with two electron dense striations (arrows) on both sides of it and a translucent torus (arrow heads) separates the matrix from both sides of the septum (bar = 0.5 μm).

Fig. 7.24 An amorphous electron dense matrix trapped in the secondary wall of the ascal base septum in Pseudoplectania nigrella (bar = 0.25 μm).


W
SW
AS
AH
18
20
21
22
23
24

CHAPTER VIII
SUMMARY AND CONCLUSIONS

The Sarcoscyphineae, including the Sarcoscyphaceae and Sarcosomataceae, has been demonstrated to be a unique group of Pezizales (Rifai, 1968). The Sarcoscyphaceae and Sarcosomataceae appear to be the most advanced groups of the Pezizales based on a number of features. These include.

1. The parasitic nature of some species. Most of the literature does not specify substrates on which the Sarcoscyphineae are found. Pithya in the Sarcoscyphaceae is restricted to conifer foliage (Dennison, 1972), and Urnula craterium (Schw.) Fries in the Sarcosomataceae has been proven to be a pathogen of oaks (Fergus, 1951). Personal observations show that Sarcoscypha occidentalis (Schw.) Sacc. is found exclusively on hickory throughout the southeastern U.S., while S. dudleyi (Peck) Sarai, U. craterium, and Galiella rufa (Schw.) Nannf. & Korf are found on various species of oaks (Quercus). Compared to the other Pezizales which are saprobic or mycorrhizal, the Sarcoscyphaceae and Sarcosomataceae with their host specificity and pathogenicity may be more evolved than the other Pezizales.

2. Ascal atructure. Chadefaud (1943) described the aporhynque type of crozier system, i.e., trailing hyphae, in the Sarcoscyphaceae and Sarcosomataceae. This character indicates that these families are highly evolved compared with the pleurorhynque type of crozier system of most of the other Pezizales.

Later, Chadefaud (1946) and Le Gal (1946a, 1946b) described the apical open rings and lens-shaped pads of the obliquely positioned opercula. However, Eckblad (1968) thought that the apical open ring was an artifact of fixation. Ultrastructural studies showed that the operculum of the Sarcoscyphaceae was eccentric and therefore different from that of the other Pezizales, including the Sarcosomataceae with a non-eccentric operculum (Samuelson, 1975, Samuelson et al., 1980). Eccentric asci of the Sarcoscyphaceae appear to be more evolved than non-eccentric asci of the other Pezizales.

3. Nuclear condition of spores. Based upon his discovery (Berthet, 1964) of multinucleate spores of the Sarcoscyphaceae and Sarcosomataceae, Berthet (1966) considered these two families to be more advanced than those families of Pezizales in which spores are uninucleate. Eckblad (1968) considered that families with uninucleate and multinucleate spores were examples of parallel evolution. However, because only Morchellaceae, Helvellaceae, Sarcoscyphaceae, and Sarcosomataceae have multinucleate

spores in the Pezizales, this feature appears to be a derived character.

4 Apothecial pigmentation The most elaborate carotenoid pigments in the Pezizales have been found in the Sarcoscyphaceae (Arpin, 1968). Arpin suggested that the larger amount of the complicated pigments a genus contained, the more advanced was the genus. From these data, he concluded that Cookeina was more advanced than Phillipsia and, in turn, Phillipsia was more advanced than Sarcoscypha. He also concluded that the Sarcosomataceae with dark colored apothecia had another pigment system and evolved parallel with the Sarcoscyphaceae.

5 Spore symmetry. Spores of the Pezizales are usually symmetrical and either globose or elliptical. However, since both the Sarcoscyphaceae and Sarcosomataceae have inequilateral spores, this characteristic is considered to be derived.

6. Structure of spore walls. Spore wall ontogeny investigated in the Sarcoscyphaceae (Chapter II-III, Fig 8.1) and Sarcosomataceae (Chapter IV VI, Fig 8.2) shows that at the ultrastructural level spore wall formation is not reliable for studying phylogenetic relationships in the Pezizales due to inconsistencies However, fine structures of the spore wall may provide useful information for resolving some taxonomic problems In general, four types of spore walls can be distinguished: smooth secondary wall,

ornamented secondary wall, smooth wall with an exposed apiapore, and ornamented primary wall. I considered a smooth secondary wall to be a primitive trait and that ornamented secondary walls and loss of secondary walls to be more evolved. The ornamented primary wall appears to be the most highly evolved. Cyanophilic spore ornements originating from the secondary wall commonly occur in the other Pezizales but cyanophobic spore markings composed of the primary wall are only found in the Sarcoscyphaceae. Therefore, the cyanophobic character is considered derived while the cyanophilic feature is conserved.

7 Septal structure. Septal pore plugs of vegetative hyphae in the Sarcoscyphaceae are usually fan shaped matrices with an electron translucent torus on each side and are associated with globose Woronin bodies. The septal structure in the paraphyses of Wynnea americana Thaxter, however, is a laminated matrix. Septal structures in ascogenous hyphae and asci of the Sarcoscyphaceae are fan-shaped matrices with electron dense striations on both sides.

Septal structures of vegetative hyphae in the Sarcosomataceae are either fan-shaped matrices as found in most of the Sarcoscyphaceae, or laminated dumbbell-shaped matrices with an electron translucent torus on each side. Woronin bodies associated with vegetative septal pore plugs range in shape from globose, hexagonal, short cylindrical,

to long cylindrical. In reproductive hyphae, pore plugs found in U. *craterium* are dumbbell-shaped matrices with V-shaped striations and a distinct electron translucent torus. In G. *rufa*, septal structures in asci are similar to those of U. *craterium*, but the laminations and torus are indistinct.

Based upon the septal structural data (Chapter VII), the Sarcoscyphaceae have almost identical pore plugs in asci as those of *Mycolachnea*, *Trichophaea* (Wu, 1991), and *Genea* (Li and Kimbrough, 1994) (Lachneae) in the Otideaceae. The Sarcosomataceae have apical septal pore plugs which are similar to that of *Geopyxis* (Kimbrough and Gibson, 1990) and some of the Aleurieae (Otideaceae) (Kimbrough and Curry, 1986a, 1986b; Wu, 1991). These data strongly support that the Sarcoscyphaceae and Sarcosomataceae are related to the tribes Aleurieae and Otideae of the Otideaceae. The long cylindrical Woronin bodies associated with vegetative septa also connect the Sarcosomataceae to the Morchellaceae and Helvellaceae (Kamaletdinova and Vasilyev, 1983; Kimbrough, 1991).

8. Systematic and phylogenetic conclusions. The Sarcoscyphaceae is a family with multiple convergence based upon apothecial morphology, spore wall ontogeny, and septal structures. *Wynnea* has darker-colored, clustered, ear shaped apothecia while *Sarcoscypha* and *Phillipsia* possess bright-colored solitary discoid-shaped apothecia. Septal

structures of Sarcoscypha and Phillipsia are similar to those of the Otideaceae. Wynnea also has vegetative septal structures different from those of the Otideaceae. Spore wall formation in this family shows many variations (Fig. 8.1). Sarcoscypha species can have spores with a smooth secondary wall (S. dudleyi) or smooth spores with an exposed epispore (i.e. without a secondary wall layer as in S. occidentalis and S. coccinea). The latter is not unlike that of many other Pezizales, including the Sarcosomataceae (Fig. 8-2). Because of cyanophobic spore markings, spore wall formation of Phillipsia and Wynnea is very different from that of the other Pezizales. Therefore, Sarcoscypha, Phillipsia, and Wynnea may have evolved recently, from different ancestors. The Sarcosomataceae are more heterogeneous than the Sarcoscyphaceae based on different types of Woronin bodies and septal structures (Chapter VII).

The Sarcoscyphaceae and Sarcosomataceae are considered highly evolved based upon the unique features discussed above. However, the origin of these families is still unclear when using these characters alone. Le Gal (1946b) theorized a phylogenetic scheme in which the suboperculate Discomycetes were derived from the inoperculate group. This scheme was questioned by Eckblad (1968). Later, Kimbrough (1994) proposed another phylogenetic scheme based upon septal structures in which the Sarcoscyphineae were derived from the Otideaceae (Pezizales). So far, the Pezizaceae are

known to have the simplest septal structures (Curry and Kimbrough, 1983). If we assume that the operculate Discomycetes are derived from the inoperculate Discomycetes, Pezizaceae is the family of Pezizales that is likely the most closely related to the inoperculate Discomycetes. Landvik et al (1993) compared 18S rDNA sequences in a number of Discomycetes and found that Pezizа was more closely aligned to the inoperculate Discomycetes than to Gyromitra (Helvellaceae), Inermisia (Otideaceae), and Plectenia (Sarcosomataceae) (= Pseudoplectenia in this study).

Since the Sarcoscyphaceae have almost identical septal structures to those of Lachnaea, there are reasons to support the idea that the Sarcoscyphaceae are more evolved than Lachnaea. First, the suboperculа of the Sarcoscyphaceae are more highly modified than the opercule of the Lachnaea. Inequilateral spores of the Sarcoscyphaceae have evolved simultaneously with the suboperculate sacci for adaptation of spore discharge (Buller, 1934). Spore markings of the primary wall are considered more advanced than the secondary wall spore ornaments of the Lachnaea. Multinucleate spores indicate that this family is more advanced, and also suggest that the Sarcoscyphaceae are related to the Morchellaceae and Helvellaceae. Septal data supports the idea that the Sarcoscyphaceae is evolved parallel with the Morchellaceae

and Helvellaceae from the Otideaceae (Fig. 8.3). Many of the Otideaceae possess simple carotenoid pigments (Arpin, 1968) which could serve as precursors of more complicated carotenoids found in the Sarcoscyphaceae. However, with several unique characteristics, the Sarcoscyphaceae are possibly more advanced than the Morchellaceae and Helvellaceae. The molecular data of ITS2 bp of rDNA done by Momol (1992) also showed that Urnula was more closely related to Otidea (Otideaceae) than to other Pezizales. Landvik et al. (1993) compared 18S rDNA sequences of various Discomycetes and found Plectania (Sarcosomataceae) (= Pseudoplectania in this study) was closely related to Gyromitra (Helvellaceae), and to Inermisia (Otideaceae). These molecular data support the concept that the Sarcosomataceae have a close affinity to the Helvellaceae and Otideaceae. A number of unique features discussed at the beginning of this chapter also indicate that the Sarcosomataceae is one of the most highly evolved families in the Pezizales. However, because the Sarcosomataceae do not have suboperculate asci (Samuelson, 1975; Samuelson et al., 1980, Bellemere et al., 1990), this family should be pulled out of the Sarcoscyphineae, provided the suboperculate ascus is retained as the main character for this suborder. Even though they may be excluded from the Sarcoscyphineae, the Sarcosomataceae would clearly represent another family in the Pezizales characterized by

pathogenicity to higher plants, an sporhynque type of crozier, inequilateral spores, and gelatinous apothecia.

Based upon the data obtained in this study and other, especially those providing molecular (Momol, 1992, Landvik et al., 1993) and septal data (Kimbrough, 1994), the Sarcoscyphaceae and Sarcosomataceae are concluded to be the most highly evolved families in the Pezizales and closely related to each other. They also appear to be derived from various tribes of the Otidaceae, have a close affinity to the Helvellaceae and Morchellaceae, and appear to represent the most highly evolved group in the phylogenetic scheme of Discomycetes.

Figura 8.1 Comparativa spora ontogeny in taxa of Sarcoscyphaceae

a: outar investing membrane

b: inner aporoplasma membrane

c: parisporic sac

d: primary wall

e: condensed primary wall material

f: epispore precursors

g: epispore

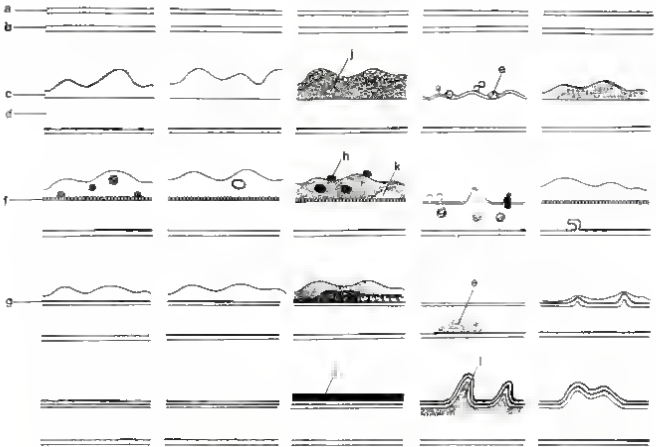
h: condensed secondary wall material

i: secondary wall

j: lacunae

k: translucent condensed secondary wall material

l: rib or ornament of the primary wall


a
b
c
d
f
g
l
e
h
k
e
l
Sarcoscypha occidentalis
Sarcoscypha coccinea
Sarcoscypha dudleyi
Phillipsia domingensis
Wynnea americana

①

Figure 8.2 Comparative spore ontogeny in taxa of Sarcosomataceae.

a: outer investing membrane

b: inner aporoplasma membrane

c: periaporic sac

d: primary wall

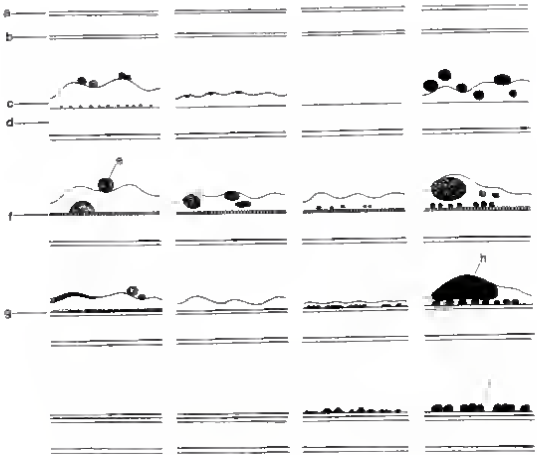
e: condensed granules

f: apiapore precursors

g: apiapore

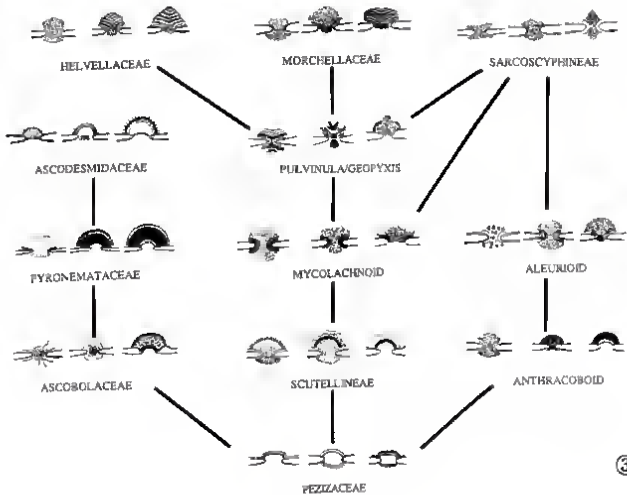
h: condensed secondary wall material

i: secondary wall


a
b
c
d
e
f
g
h
Urnula craterium
Pseudoplectania nigrella
Plectania sanfeldii
Galiella rufa

②

Figure 8.3 A phylogenetic scheme of Sarcoscyphinae and other families in Pezizales based upon septal structures in asci.


HELVELLACEAE
MORCHELLACEAE
SARCOSCYPHINEAE
ASCODESMIDACEAE
PULVINULA/GEOPYXIS
PYRONEMATACEAE
MYCOLACHNOID
ALEURIOID
ASCOBOLACEAE
SCUTELLINEAE
ANTHRACOBOID
PEZIZACEAE

③

BIBLIOGRAPHY

Arpin, N. 1968. Les carotenoides de discomycètes: Essai chimiotaxonomique. Thèse 527. Univ. Lyon. Villeurbanne, France. 170 pp.

Beckett, A. 1981. Ascospore formation: pp. 107-129. In: The Fungal Spore: Morphogenetic Controls. Edited by G. Turian, and H. R. Hohl. Academic Press. New York

Beckett, A., Illingworth, R. F., and Rose, A. H. 1973. Ascospore wall development in Saccharomyces cerevisiae. Journal of Bacteriology 113: 1054-1057

Bellemère, A., Malherbe, M. C., Chacun, H., and Melendez-Howell, L. M. 1980. L'Étude ultrastructurales des asques et des ascospores de l' Urnula helvelioides Donadini, Berthet et Astier et les concepts d'asque subopercule et de Sarcosomatacées. Cryptogamie, Mycol. 11: 203-238.

Benny, G. L., and Samuelson, D. A. 1980. Ultrastructure of septa in the vegetative hyphae of Chaetomidium arxii and Philippsia dominganeis (Ascomycotina). Mycologia 72: 836-840

Berkeley, M. J. 1867. On some new fungi from Mexico. J. Linn. Soc. Bot. 9: 423-425

Berkeley, M. J. 1881. Australian fungi II. Received principally from Baron F. von Mueller. J. Linn. Soc. Bot. 18: 388-389

Berthet, P. 1964. Essai biotaxonomique sur des Discomycètes. Ph.D. thèse, Université Lyon. France. 157 pp.

Berthet, P. 1966. Essai d'une Phylogénie des Discales. C. R. 4e Cong. des Mycologues Européens, Barcelone

Boudier, J. L. E. 1879. On the importance that should be attached to the dehiscence of asci in the classification of the Discomycetes. Grevillea 8: 45-49.

Boudier, J. L. E. 1885. Nouvelle classification naturelle des discomycetes charnus connus généralement sous le nom de pezizes. Bull. Soc. Mycol. Fr. 1: 91-120.

Brenner, D. M., and Carroll, G. C. 1968. Fine-structural correlates of growth in hyphae of Ascodesmis sphaerospora. Journal of Bacteriology 95: 658-671.

Brummelen, J. Van. 1975. Light and electron microscopic studies of the ascus top in Sarcoscypha coccinea. Persoonia 8: 259-271.

Buller, A. H. R. 1934. The heliotropism of the ascl and the discharge of the spores in Ascobolus, Cillaria scutellata, Aleuria vesiculosa, the Morchellaceae, and other Discomycetes. Researches on Fungi 6: 264-324.

Carroll, G. C. 1967. The fine structure of the ascus septum in Ascodesmis sphaerospora and Saccobolus kerverni. Mycologia 59: 527-532.

Chadefaud, M. 1943. Sur les divers types d'éléments dangeardiens des Ascomycetes, et sur la formation des asques chez la Pezize Pustularia catinus. La Revue Scientifique 81: 77-80.

Chadefaud, M. 1946. Sur les asques à nesse apicale. Bull. Sci. Bot. de France. 93: 128-130.

Clements, F. E., and Shear, C. L. 1931. The genera of fungi. 496 pp. New York.

Cooke, M. C. 1892. Handbook of Australian Fungi. Williams and Norgate Pub. London. 458 pp.

Crouan, P. L., and Crouan, H. M. 1857. Note sur quelques Ascobolus nouveaux et sur une espece nouvelle de Vibrissea. Ann. Sci. Nat. (Bot.) IV 7: 173-178.

Curry, K. J., and Kimbrough, J. W. 1983. Septal structures in apothecial tissues of the Pezizaceae (Pezizales, Ascomycetes). Mycologia 75: 781-794.

Davidson, R. W. 1950. Urnula craterium is possibly the perfect stage of Strumella coryneoidea. Mycologia 42: 735-742.

Danielson, W. C. 1969. Central American Pezizales. III. The genus Phillipsia. Mycologia 61: 289-304.

Denison, W. C. 1972. Central American Pezizales. IV. The genera Sarcoscypha, Plthya, and Nanoscypha. Mycologia 64: 609-623.

Dennis, R. W. G. 1960. British Cup Fungi and Their Allies. An introduction to the Ascomycetes. The Ray Soc., London. 280 pp.

Dennis, R. W. G. 1968. British Ascomycetes. J. Cramer-Lehre. 455 pp.

Donadini, J. C., Chacun, H., Malherbe, M. C., and Ballemère, A. 1989. L'ultrastructure des asques et des ascospores du Pseudopithyella minuscula (Ascomycètes Pézizales Sarcosomataceae). Cryptogamie, Mycol. 10: 283-304.

Dyby, S., and Kimbrough, J. W. 1987. A comparative ultrastructural study of ascospore ontogeny in selected species of Peziza (Pezizales, Ascomycetes). Bot. Gaz. (Crawfordsville) 148: 283-296.

Eckblad, F. E. 1968. The genera of the operculate Discomycetes. A re-evaluation of their taxonomy, phylogeny and nomenclature. Nytt Mag. Bot. 15: 1-191.

Eckblad, F.-E. 1972. The suboperculate ascus. Persoonia 6: 439-443.

Eriksson, O. E., and Hawksworth, D. L. 1993. Notes on ascomycete systematics-Nos. 1418-1529. Systema ascomycetum 11: 206-207.

Fergus, C. L. 1951. Strumella canker on bur oak in Pennsylvania. Phytopathology 41: 101-103.

Fries, E. M. 1822. Systema mycologicum. 2: 1-620.

Fries, E. M. 1849. Summa vegetabilium Scandinaviae. A. Bonnier, Uppsala. 572 pp.

Fuckel, L. 1870. Symbolae mycologicae. Beiträge zur Kenntniss der rheinischen Pilze. Jahrb. Nass. Ver. Nat. 1-459.

Gibson, J. L., and Kimbrough, J. W. 1988a. Ultrastructural observations on Helvellaceae (Pezizales). I. Ascosporogenesis of selected species of Helvella. Can. J. Bot. 66: 771-783.

Gibson, J. L., and Kimbrough, J. W. 1988b. Ultrastructural observations on Helvellaceae (Pezizales). II. Ascoporogenesis of Gyromitre ceculenta. Can. J. Bot. 66: 1743-1749.

Harrington, F. A. 1990. Sarcoscyphe in North America (Pezizales, Sarcoscyphaceae). Mycotaxon 38: 417-458.

Heald, F. D., and Wolf, F. A. 1910. The structure and relationship of Urnula gesatar. Bot. Gaz. 49: 182-188.

Höhnel, F. von. 1917. Mycologische fragmente, CXX CXC. Ann. Mycol. 15. 292-383.

Kameletdinowa, F. I., and Veselivev, A. E. 1983. Cytology of Discomycetes. NAUKA Pub., Kazakh SSR. 174 pp.

Kanouse, B. B. 1948. The genus Plectania and its segregates in North America. Mycologie 40: 482-497.

Kimbrough, J. W. 1991. Ultrastructural observations on Helvellaceae (Pezizales, Ascomycetes). V. Septal structures in Gyromitra. Mycol. Res. 95: 421-426.

Kimbrough, J. W. 1994. Septal ultrastructure and Ascomycete systematics. In: D. L. Hawksworth, ed. First International Workshop on Ascomycete Systematics. Plenum Pub. Co., Ltd., London, United Kingdom (in press).

Kimbrough, J. W., and Curry, K. J. 1985. Septal ultrastructure in the Ascobolaceae (Pezizales, Discomycetes). Mycologie 77: 219-229.

Kimbrough, J. W., and Curry, K. J. 1986a. Septal structures in apothecial tissues of the tribe Aleurieae in the Pyronemataceae (Pezizales, Ascomycetes). Mycologie 78: 407-417.

Kimbrough, J. W., and Curry, K. J. 1986b. Septal structures in apothecial tissues of taxa in the tribes Scutalinieae and Sowerbyelleae (Pyronemataceae, Pezizales, Ascomycetes). Mycologie 78: 735-743.

Kimbrough, J. W., and Gibson, J. L. 1989. Ultrastructural observations on Helvellaceae (Pezizales, Ascomycetes). III. Septal structures in Helvella. Mycologie 81: 914-920.

Kimbrough, J. W., and Gibson, J. L. 1990. Ultrastructural and cytological observations of apothecial tissues of Geopyxis carbonaria (Pezizales, Ascomycetes). Can. J. Bot. 68: 243-257.

Kimbrough, J. W., and Wu, C.-G. 1990. Ultrastructural observations on Helvellaceae (Pezizales, Ascomycetes). IV. Ascospore ontogeny in selected species of Gyromitra subgenus Discina. Can. J. Bot. 68: 317-328.

Kimbrough, J. W., Wu, C.-G., and Gibson, J. L. 1991. Ultrastructural evidence for a phylogenetic linkage of the truffle genus Hydnobolites to the Pezizaceae (Pezizales, Ascomycetes). Bot. Gaz. 152: 408-420.

Kobayasi, Y. 1937. On the gelatinous cup fungi, Sulgaria-group. Jour. Japan. Bot. 13: 511-520.

Korf, R. P. 1949. Wynnea americana. Mycologia 41: 649-651.

Korf, R. P. 1954. A revision of the classification of operculate Discomycetes (Pezizales). VIII. Congr. Int. Bot., Rapp. & Comm. 18-20: 80.

Korf, R. P. 1957. Two bulgarioid genera: Galiziia and Plectania. Mycologia 49: 107-111.

Korf, R. P. 1958. Japanese Discomycete notes I-VIII. Sci. Rep. Yokohama Nat. Univ. II, 7: 7-35.

Korf, R. P. 1970. Nomenclature notes VII. Family and tribe names in Sarcoscyphineae (Discomycetes) and a new taxonomic disposition of the genera. Taxon 19: 782-786.

Korf, R. P. 1973. Discomycetes and Tuberales: pp. 249-319. In: The fungi IVa, an advanced treatise. Edited by C. G. Ainsworth, K. Sparrow, and A. S. Sussman. Academic Press, New York.

Kotlaba, F., and Pouzar, Z. 1964. Preliminary results on the staining of spores and other structures of Homobasidiomycetes in cotton blue and its importance for taxonomy. Feddad Rpert. Spec. Nov. Regni Veg. 69: 131-142.

Kupfer, E. M. 1902. Studies on Urnula and Geopyxis. Bull. Torrey Botan. Club. 29: 137-144.

Landvik, S., Erikssen, O. E., Gargas, A., and Gustafsson, P. 1993. Relationships of the genus Neolecta (Neolectales ordo nov., Ascomycotina) inferred from 18S rDNA sequences. Systema Ascomycetum 11: 107-118.

Le Gal, M. 1946a. Mode de dehiscence des asques du point de vue phylogenetique. C. R. Acad. Sci. Paris, 222: 755-757.

Le Gal, M. 1946b. Les Discomycètes eubopercules. Bull. Soc. Mycol. Fr. 62: 218-240.

Le Gal, M. 1947. Recherches sur les ornamentations sporales des Discomycètes operculés. Ann. Sci. Nat., Bot. Biol. Veg. 7: 193-297.

Le Gal, M. 1953. Les Discomycetes de Madagascar. Museum National d'Histoire Naturelle, Paris. 465 pp.

Le Gal, M. 1958. Le genre Melastiza Boudier. Bull. Soc. Mycol. France. 74: 149-154.

Li, L.-T., and Kimbrough, J. W. 1994. Ultrastructural evidence for a relationship of the truffle genus Genea to Otideaceae (Pezizales). Int. J. Plant Sci. 155: (in Press).

Melendez-Howell, L. M., Chacun, H., Malherbe, M. C., and Bellemère, A. 1980. Étude ultrastructurale de l'apex de l'asque et de la perol des ascospores dans le genre Pithya Fuck. (Ascomycetes, Pezizales, Sarcosomataceae). Anales Inst. Biol. Univ. Nac. Auton. México, Ser. Bot. 60: 9-20.

Markua, E. 1973. Ultrastructure of the ascospore wall in Pezizales (Ascomycetes)-I. Ascodesmis microscopica (Crouan) Seaver and A. nigricans van Tieghem. Persoonia 7: 351-366.

Markua, E. 1974. Ultrastructure of the ascospore wall in Pezizales (Ascomycetes) II. Pyronemataceae sensu Eckblad. Persoonia 8: 1-22.

Markus, E. 1975. Ultrastructure of the ascospore wall in Pezizales (Ascomycetes)-III. Otideaceae and Pezizaceae. Persoonia 8: 227-247.

Markus, E. 1976. Ultrastructure of the ascospore wall in Pezizales (Ascomycetes) IV. Morchellaceae, Helvellaceae, Rhizinaceae, Thelebolaceae, and Sarcoscyphaceae. General discussion. Persoonia 9: 1-38.

Mims, C. W., Richardson, E. A., and Kimbrough, J. W. 1990. Ultrastructure of ascospore delimitation in freeze substituted samples of Ascodesmis nigricans (Pezizales). Protoplasma 156: 94-102.

Momol, E. A. 1992. Nuclear ribosomal DNA sequence analysis in molecular systematics of Pezizales (Ascomycetes). Ph.D. Dissertation. Plant Pathology Department, University of Florida, Gainesville. 90 pp.

Moser, M. 1963. Ascomycetes. In: H. Gams, Kleine Kryptogamenflora 2a, 147 pp. Gustav Fischer Verlag, Stuttgart.

Nannfeldt, J. A. 1949. Contributions to the Mycoflora of Sweden. 7. A new winter Discomycete, Urnula hiemalis Nannf. n. sp., and a short account of the swedish species of Sarcoscyphaceae. Svensk Bot. Tidskr. 43: 466-484.

Pfister, D. H. 1970. A histochemical study of the composition of spore ornamentations in operculate Discomycetes. Mycologia 62: 234-237.

Pfister, D. H. 1979. A monograph of the genus Wynnea (Pezizales, Sarcoscyphaceae). Mycologia 71: 144-159.

Rehm, H. 1896. Die Pilze Deutschlands, Oesterreichs und der Schweiz. III. Abtheilung: Ascomyceten: Hysteriaceen und Discomyceten. in: Rab. Krypt-Fl. 1. Verlag Kummer, Leipzig.

Rifai, M. A. 1968. The Australasian Pezizales in the herbarium of the Royal Botanic Gardens, Kew. Verh. K. Ned. Akad. Wet. Afd. Natuurkd., Reeks 2, 57: 1-295.

Saccardo, P. A. 1884. Conspectus generum Discomycetum hucusque cognitorum. Bot. Cbl. 18: 213-220, 247-256.

Samuelson, D. A. 1975. The apical apparatus of the suboperculate ascus. Can. J. Bot. 53: 2660-2679.

Samuelson, D. A., Benny, G. L., and Kimbrough, J. W. 1980. Asci of the Pezizales. VII. The apical apparatus of Galiella rufa and Sarcosoma globosum: reevaluation of the suboperculate ascus. Can. J. Bot. 58: 1235-1243.

Schweinitz, L. D. von. 1832. Synopsis fungorum in America boreali media degentium. Trans. Am. Phil. Soc. II. 4: 141-316.

Seaver, F. J. 1928. The North American Cup-fungi (Operculates). (pub. by author). New York. 377 pp.

Seaver, F. J. 1942. The North American Cup-fungi (Operculates). Suppl. ed. (pub. by author). New York. 284 pp.

Thaxter, R. 1905. Contributions from the cryptogamic laboratory of Harvard University. LX. A new American species of Wynnea. Bot. Gaz. 39: 241-247

Wergin, W. P. 1973. Development of Woronin bodies from microbodies in Fusarium oxysporum f. sp. lycopersici. Protoplasma 76: 249-260

Wolf, F. A. 1959. Mechanism of apothecial opening and ascospore expulsion by the cup-fungus Urnula craterium. Mycologia 50: 837-843.

Wu, C.-G. 1991. Ultrastructural investigation of Humariaceae (Pezizales, Ascomycetes): spore ontogeny, septal structure, and phylogeny. Ph.D. Dissertation. Plant Pathology Department, University of Florida, Gainesville. 262 pp.

Wu, C.-G., and Kimbrough, J. W. 1991. Ultrastructural investigation of Humariaceae (Pezizales, Ascomycetes). II. Ascosporogenesis in selected genera of the Cilliariae. Bot. Gaz. 152: 421-438

Wu, C.-G., and Kimbrough, J. W. 1992a. Ultrastructural investigation of Humariaceae (Pezizales, Ascomycetes). III. Ascosporogenesis of Mycolachnea hemiphaerica (Tribe Lachnaea). Int. J. Plant Sci. 153: 128-135.

Wu, C.-G., and Kimbrough, J. W. 1992b. Ultrastructural studies of ascosporogenesis in Ascobolus immersus. Mycologia 84: 459-466

Wu, C.-G., and Kimbrough, J. W. 1993. Ultrastructure of ascospore ontogeny in Aleuria, Octospora, and Pulvinula (Otideaceae, Pezizales). Int. J. Plant Sci. 154: 334-349

BIOGRAPHICAL SKETCH

Li-Tzu Li was born in Tainan, Taiwan, Republic of China, on June 30, 1966 and grew up in Sanchung, Taipei. She finished her primary school and junior high school education in 1978 and 1981, respectively. In June of 1984, she graduated from Ching Mei Girls' Senior High School, Taipei. Later the same year, she entered National Chung-Hsing University in Taichung majoring in plant pathology. She received her B. S. degree in 1988. Under Dr. W. H. Hsieh's guidance she studied mycology and received her M. S. degree in plant pathology there in 1990. In August of 1990, she continued her graduate studies toward the Ph.D. degree in the Plant Pathology Department, University of Florida. She was married to Changshiann Wu in July 1992.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James W. Kimbrough, Chairman
Professor of Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Raghavan Charudattan
Professor of Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Francis W. Zettler
Professor of Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Henry C. Aldrich
Professor of Microbiology and Cell Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Saeedur R. Khan
Associate Professor of
Pathology and Laboratory
Medicine

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

April, 1994

Jack L. Fry
Dean, College of Agriculture

Dean, Graduate School